STUDY OF SERUM FIBROBLAST GROWTH FACTOR 23 (FGF23) LEVELS IN CHRONIC KIDNEY DISEASE

Dissertation Submitted for

M.D DEGREE BRANCH - XIII

[BIO CHEMISTRY]

DEPARTMENT OF BIOCHEMISTRY

K.A.P.V GOVT. MEDICAL COLLEGE,

TRICHY.

THE TAMILNADU DR.M.G.R MEDICAL UNIVERSITY,

CHENNAI

APRIL - 2016

CERTIFICATE

This is to certify that dissertation titled"**STUDY OF SERUM FIBROBLAST GROWTH FACTOR-23(FGF-23) LEVELS IN CHRONIC KIDNEY DISEASE**" is a bonafide work done by **Dr.PRIYA .A** under my guidance and supervision in the Department of Biochemistry, K.A.P.V Govt. Medical College, Trichy during her post graduate course from 2013 to 2016.

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DECLARATION

I, **Dr.PRIYA.A** hereby solemnly declare that the dissertation titled"**STUDY OF SERUM FIBROBLAST GROWTH FACTOR-23(FGF-23) LEVELS IN CHRONIC KIDNEY DISEASE**"was done by me at K.A.P.V Govt Medical College & MGMGH, Trichy under the Supervision and Guidance of my Professor and H.O.D **Dr.K.Nirmaladevi.D.C.H.,M.D(Bio).,** . This dissertation is submitted to Tamil Nadu Dr. M.G.R Medical University, towards partial fulfillment of requirement for the award of M.D. Degree (Branch –XIII) in Biochemistry.

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This is to certify that the project work titled **Study of serum fibroblast growth factor 23(FGF-23) levels in chronic kidney disease** proposed by **Dr.A.Priya** part of fulfillment of M.D/M.S course in the subject of **Biochemistry** for the year **2013-2016** by The Tamilnadu Dr.MGR Medical University has been cleared by the ethics committee.

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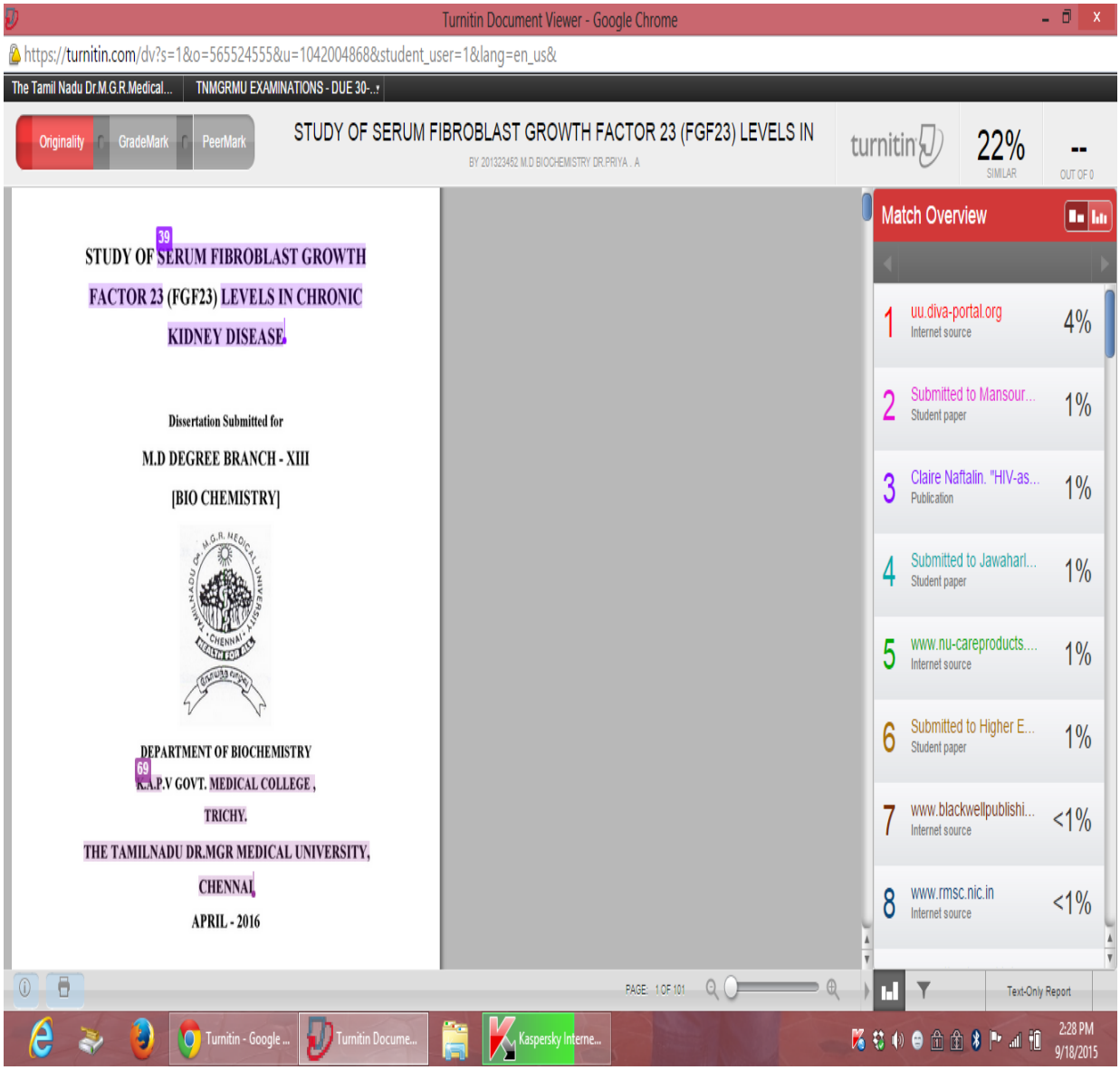

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INTRODUCTION

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CONTENTS

ABBREVIATIONS

ADHR	Autosomal Dominant Hyperphosphatemic Rickets
ADPCKD	Autosomal Dominant Polycystic Kidney Disease
CAPD	Continuous Ambulatory Peritoneal dialysis
CKD	Chronic Kidney Disease
CKD-EPI	Chronic Kidney Disease – Epidemiology Collaboration equation
CKD-MBD	Chronic Kidney Disease – Mineral and Bone Disorder
CVD	Cardio Vascular Disease
DM	Diabetes Mellitus
EGF	Epidermal Growth Factor
eGFR	Estimated Glomerular Filtration Rate
ELISA	Enzyme-linked Immunosorbent Assay
ESRD	End Stage Renal Disease
ERK	Extracellular Signal Regulated Kinase
FBG	Fasting Blood Glucose
FGF23	Fibroblast Growth Factor 23
FGFR	FGF Receptor
GALNT3	N-acetylgalactosaminyltransferase3
GFR	Glomerular Filtration Rate
HRP	Horse Radish Peroxidase
HGF	Hepatocyte Growth Factor
HT	Hypertension

ICAM-1	Intercellular Adhesion Molecule-1
IL-1β	Interleukin- 1 Beta
LDL-C	Low density lipoprotein – Cholesterol
LVH	Left ventricular Hypertrophy
MDRD	Modification of Diet in Renal Disease
MHC	Major histocompatability complex
NICE	National Institute of Clinical Excellence
NKF-KDOQI	National Kidney Foundation - Kidney Disease Outcome Quality Initiative
NO	Nitric Oxide
NPT	Sodium-dependent Phosphate cotransporter
PDGF	Platelet Derived Growth Factor
PTH	Parathormone
RRT	Renal Replacement Therapy
TGF-β	Transforming Growth Factor-β
TNF-α	Tumour Necrosis Factor- alpha
TIS	Tubulointerstitial scarring
SEEK	Screening and Early Evaluation of Kidney Disease
VDRE	Vitamin D responsive element
VEGF	Vascular Endothelial Growth Factor
XLH	X-linked Hypophosphatemic rickets

INTRODUCTION

INTRODUCTION

Chronic kidney disease (CKD) is a major public health problem all over the world. CKD is the 12th leading cause of death and 17th cause of disability worldwide[1]. CKD leads to poor outcomes like End Stage Renal Disease (ESRD), Cardio Vascular Disease (CVD) and Premature Death[2].

Included in CKD is a spectrum of many pathophysiological processes associated with abnormal kidney function and a progressive decline in Glomerular Filtration Rate (GFR)[3]. The definition of CKD is based on the extent of kidney damage and level of kidney function—irrespective of the type of kidney disease.

Glomerular filtration rate (GFR) is the primary measure of kidney function. Normal GFR is 120ml/min and it varies with age, sex and race. Patients with CKD have a decreased GFR and they develop mineral metabolic disturbance such as hyperphosphatemia even before the diagnosis of CKD is made.

Fibroblast growth factor (FGF)-23 is a newly recognized phosphatonin secreted by the osteocytes which acts as a key regulator of serum phosphate levels in CKD. It initiates secondary hyperparathyroidism in patients with CKD. FGF-23 together with its co-receptor Klotho binds to specific FGF Receptor (FGFR) in the target organs – kidneys and parathyroid glands. FGF-23 induces phosphaturia and causes suppression of Vit.D production in the kidney[4].

Serum FGF-23 levels rise progressively as renal function decreases and increased levels of FGF-23 is an independent predictor of CKD progression, cardiovascular morbidity and mortality [5].

FGF-23 levels are increased even in the early stages of CKD while creatinine based estimations of GFR which are used to assess the kidney function do not detect early CKD[6]. So measurement of serum FGF-23 levels could therefore have a definitive advantage in the early diagnosis and in predicting the progression of CKD and therapeutic strategies to reduce serum FGF-23 levels could be of great benefit to the patient in terms of improved survival[7].

Hence in the present study, the serum FGF-23 levels were estimated in patients with different stages of CKD and comparison of FGF-23 levels with estimated Glomerular Filtration Rate (eGFR) and kidney size were made in various stages of CKD.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The kidneys are organs that play an important role to maintain the homeostatic mechanisms of the body and a decrease in renal function correlates strongly with morbidity and mortality[8].

FUNCTIONS OF THE KIDNEY :

1. Filtration – Preparation of Ultrafiltrate
2. Reabsorption of Aminoacids, Glucose, Proteins and Electrolytes
3. Homeostasis of Extracellular fluid, Blood pressure, Acid-base and electrolyte balance
4. Metabolic functions:

   Synthesis of Ammonia, Glutathione, Gluconeogenesis,

   Catabolism of Hormones
5. Endocrine Functions:

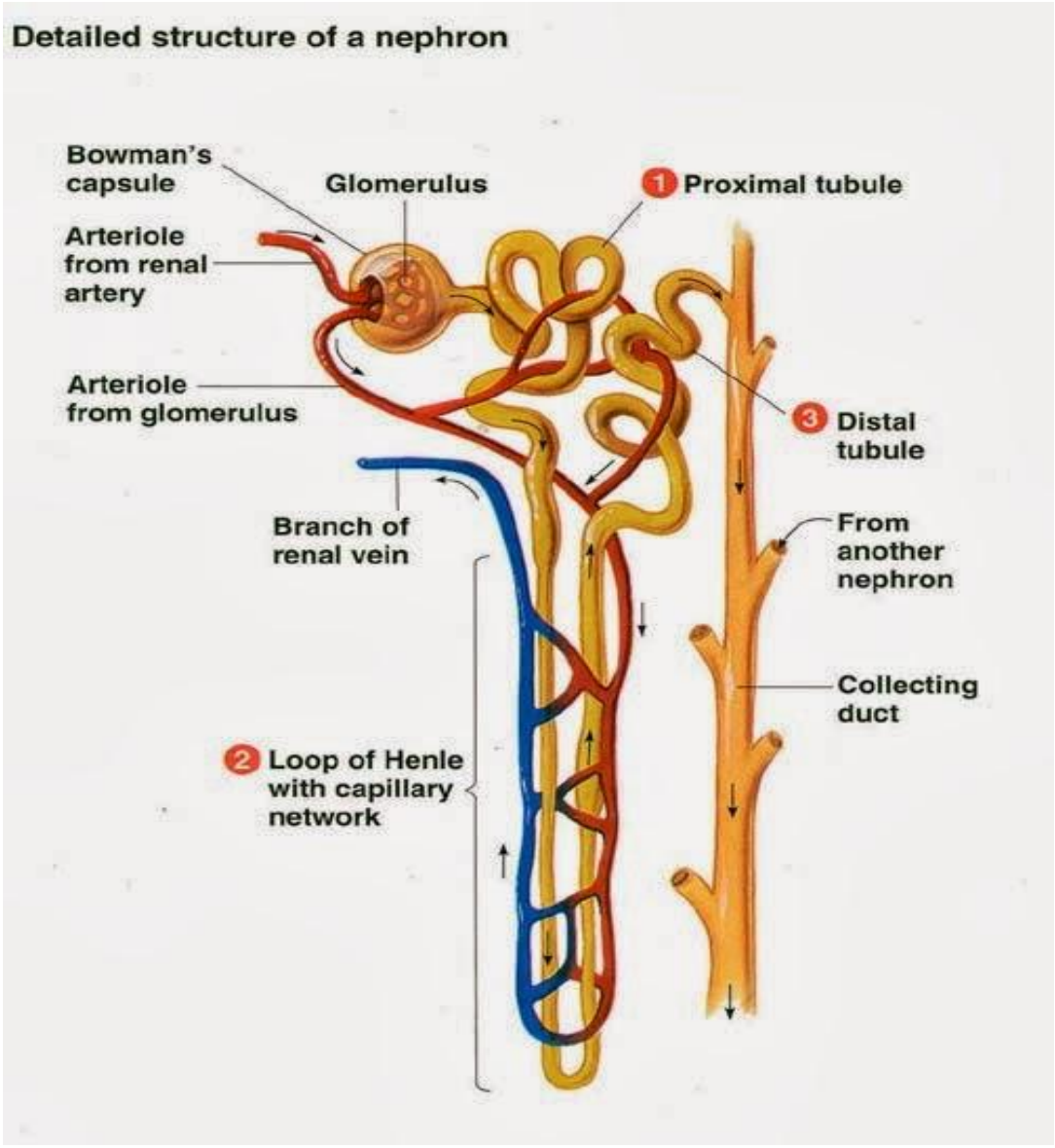
   Erythropoietin synthesis

   Vitamin D3 activation

   Renin release

Nephron is the functional unit of the kidney. About 600,000 – 1.5 million nephrons are present in each kidney. Nephron consists of a glomerulus, proximal convoluted tubule, loop of Henle, distal convoluted tubule and collecting duct. The number of nephrons in an individual determines the individual's susceptibility to kidney injury.

Fig.1 Structure of a Nephron



REQUIREMENTS FOR NORMAL RENAL FUNCTION :

1. Free flow of blood through the Glomerular Capillaries
2. Adequate functioning of glomerular filter
3. Selective reabsorbing ability of the tubules for important substances from the filtrate and to excrete other constituents into the filtrate
4. Free flow of urine formed by the nephron in the kidney into the bladder and out of the urethra[9]

Any derangement in the above said functions lead to kidney disease.

CHRONIC KIDNEY DISEASE (CKD) :

Chronic Kidney Disease (CKD) is an important health problem,emphasizing the need for early identification and treatment. The NKF-K/DOQI(National Kidney Foundation-Kidney Disease Outcome Quality Initiative) published a definition of CKD to identify the disease at its early stages.

Definition of CKD[10]:

Kidney damage for ≥ 3 months, as defined by structural or functional abnormalities of the kidney with or without decreased GFR, manifested by either pathological abnormalities or markers of kidney damage including

- abnormalities in composition of blood / urine or abnormalities in imaging tests
- GFR < 60 mL / 1.73 square metre ≥ 3 months with or without kidney damage

- Kidney Failure as defined by NKF-K/DOQI as a GFR < 15 mL /1.73 square metre.

The term kidney failure is established by End Stage Renal Disease(ESRD), defined as "Chronic Kidney Disease that has progressed so far that Renal Replacement Therapy (RRT) i.e., Dialysis and Transplantation is required to maintain life".

EPIDEMIOLOGY OF CKD[11,12] :

The overall prevalence of CKD has increased from 12% (1988 - 1994) to 40% (1999 - 2004) but has since remained stable. The largest increase occurred in stage 3 CKD from 4.5% to 6% over three time periods.

The increased prevalence may be due to several factors like

1. Metabolic Syndrome
2. Diabetes Mellitus
3. Hypertension
4. Obesity

Diabetes Mellitus is the largest single cause of advanced CKD, contributing to ~ 50% of new dialysis patients. Hypertension contributes to ~ 25% of CKD patients.

CURRENT SCENARIO IN INDIA[13] :

The prevalence of CKD in India is ~ 17.2 % based on SEEK (Screening and Early Evaluation of Kidney Disease) study in 2013. Diabetes Mellitus contributes to ~ 30% among CKD patients. As per the study, only ~ 20% of

End Stage Renal Disease (ESRD) patients were on Renal Replacement Therapy (RRT).

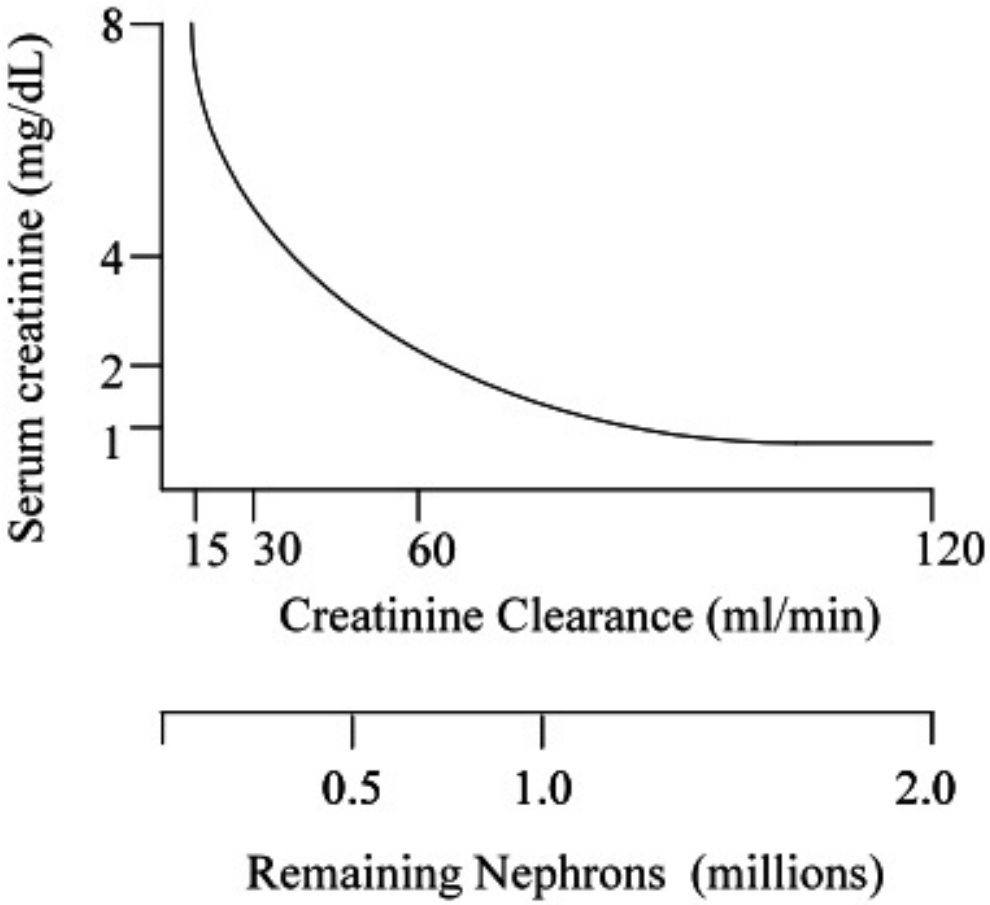
Patients with CKD are classified according to severity of the disease based on the levels of GFR.

GLOMERULAR FILTRATION RATE (GFR) :

Glomerular Filtration Rate (GFR) is the most reliable measure of the functional capacity of the kidneys. It is indicative of the number of functioning nephrons. The GFR in healthy individuals is approximately 74-129 mL/min in males and 65-123 mL/min in females.

The magnitude of GFR correlates with the surface area. The rate of formation of Glomerular filtrate depends on the balance between hydrostatic pressure and oncotic forces along the afferent arteriole and across the glomerular filter[14].

Fig.2 Relationship between Serum Creatinine and Creatinine Clearance



MAJOR INFLUENCING FACTORS ON GFR :

1. Changes in Renal blood flow
2. Changes in Glomerular Capillary Hydrostatic pressure
3. Changes in Hydrostatic pressure in Bowman's capsule
4. Changes in Concentration of plasma proteins
5. Changes in Effective Filtration Surface area

An alternative to serum creatinine is the endogenous marker cystatin C. It is a 13 kDa protein, produced by every nucleated cell. Glomerular Filtration rate chiefly determines serum cystatin C levels. Potential limitations of cystatin C as a marker of GFR are lack of standardized assays and the cost factor when compared to serum creatinine[15].

STAGING OF CKD[16] :

Among CKD patients, Staging of the disease must be assigned based on

i. Level of kidney function irrespective of etiology, according to NKF-K/DOQI CKD Classification.

CKD is divided it into 5 stages as per NKF-K/DOQI Classification and National Institute of Clinical Excellence (NICE) guidelines[17].

STAGE	DESCRIPTION	GFR(mL/min1.73 m^2)
1	Kidney damage with normal or ↑ GFR	≥ 90
2	Kidney damage with mild ↓GFR	60 – 89
3A	Moderate ↓GFR	45 – 59
3B	Moderate ↓GFR	30 – 44
4	Severe ↓GFR	15 – 29
5	Kidney failure	< 15 (or dialysis)

Staging of CKD requires continuous measure of kidney function to categorize and the cut-off levels between stages are arbitrary. Staging facilitates appropriate treatment intervention and quality improvement efforts to the evaluation and management of CKD.

The assessment of GFR can be done by either 24 hrs Creatinine Clearance or from Serum Creatinine using any one of the following formulas[18,19]

1. Modification of Diet in Renal Disease (MDRD) formula

$$eGFR = 186 \times (Pcr)^{-1.154} \times (\text{Age in years})^{-0.203}$$

- Multiply by 0.742 for women.
- Multiply by 1.21 for Blacks.
- Pcr– Plasma Creatinine in mg/dl

2. Cockcroft – Gault formula

$$\text{Estimated Creatinine Clearance} = \frac{(140\text{-Age}) \times \text{Wt in Kg}}{72 \times \text{Serum Creatinine}}$$

- Multiply by 0.85 for females

3. Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation

$$eGFR = 141 \times \min(SCr/k,1)^{\alpha} \times \max(SCr/k,1)^{-1.209} \times 0.993^{Age}$$

- Multiply by 1.018 for females
- Multiply by 1.159 for blacks
- SCr - serum creatinine (mg/dL)
- k is 0.7 for females and 0.9 for males
- α is -0.329 for females and -0.411 for males
- min indicates the minimum of SCr/k or 1
- max indicates the maximum of SCr/k or 1

NATURAL HISTORY OF CKD:

CKD significantly contributes to the morbidity and mortality. Some patients have a clear risk of progression to ESRD and for some, risk factors such as Diabetes, Hypertension and Proteinuria exist which are possible novel risk factors that cause progression of CKD[20].

The proposed natural history of CKD initially causes kidney damage which on progression leads to a much decreased renal function and ultimately, leading to ESRD[21]. Many patients with CKD, stages 3-5 progress relentlessly

to ESRD. The rate of progression of CKD varies among individuals based on the underlying pathology .

Diabetic Nephropathy is associated with much faster decline in eGFR and a much faster progression of the disease but they are more likely to die before ESRD. The absence of optimal BP control and proteinuria, indicates the renal risk inherent to the cause of CKD. As the primary diagnosis is unmodified by CKD Stage or eGFR, the underlying renal disease should be considered to assess the renal risk[22].

ETIOLOGY OF CKD[23] :

1. Diabetic Glomerulosclerosis
2. Hypertensive Nephrosclerosis
3. Glomerular disease
    - Glomerulonephritis
    - Amyloidosis, Light chain disease
    - Systemic Lupus Erythematosus, Wegener's Granulomatosis
4. Tubulointerstitial disease
    - Reflux Nephropathy (Chronic pyelonephritis)
    - Analgesic Nephropathy
    - Obstructive Nephropathy (Stones, Benign Prostatic Hypertrophy)
    - Myeloma Kidney
5. Vascular disease
    - Scleroderma
    - Vasculitis

- Ischemic Nephropathy
- Atherosclerotic renal disease

6. Cystic diseases
    - Autosomal Dominant Polycystic Kidney Disease (ADPCKD)
    - Medullary Cystic Kidney Disease

RISK FACTORS FOR CKD & ITS OUTCOMES[24]:

1. Susceptibility factors:
    - Old age
    - Family History of CKD
    - Reduction in Kidney mass
    - Low birth weight
2. Initiation factors :
    - Diabetes Mellitus
    - High Blood pressure
    - Autoimmune disorders
    - Systemic Infections
    - Urinary Tract Infection
    - Drug Toxicity
    - Urinary Stones
3. Progression Factors :
    - High Proteinuria
    - High Systolic Blood pressure
    - Poor Glycemic control in Diabetes Mellitus

- Smoking

4. End stage Factors :
    - Lower dialysis dose
    - Temporary Vascular access
    - Anemia
    - Decreased Serum Albumin levels
    - Late referral

PRESENTATION OF CKD[25] :

- Hypertension
- Urinary abnormality
- Acute Kidney Injury (due to Infection, Dehydration, Drug toxicity)
- Uremia
    - Malaise, Lethargy
    - Anorexia, Weight loss
    - Nocturia
    - Volume overload
    - Pericarditis
    - Pericardial tamponade
    - Osteomalacia
    - Bone fractures

STAGEWISE REPRESENTATION OF CKD[26] :

STAGE 1:

- Denotes kidney damage when GFR is normal or high (≥90 ml/min)
- Patients are usually asymptomatic.
- Includes patients with microalbuminuria and mild/moderate/severe proteinuria and/or those with abnormal imaging studies.

STAGE 2:

- Evidence of kidney damage with mild decrease in GFR(60-89 ml/min)
- Patients are usually asymptomatic.
- Blood Urea Nitrogen and Serum Creatinine are usually normal.
- Acid Base, Fluid and Electrolyte balance are maintained within normal limits by an adaptive increase of function in the remaining nephrons.

STAGE 3:

- Moderate decline in GFR(30-59 ml/min)
- Serum Creatinine starts to rise.
- Most patients still remain asymptomatic.
- Early symptoms include nocturia and polyuria.
- Serum Creatinine and Blood urea nitrogen are increased.
- Levels of Erythropoietin, Calcitriol and Parathormone(PTH) are usually abnormal.

STAGE 4:

- Severe fall in GFR occurs(15-29 ml/min)
- Overt Uremic symptoms develop.

- Hypocalcemia, acidosis, hyperphosphatemia & hyperkalemia occur.

STAGE 5:

- GFR falls below15 ml/min
- Worsening of all the symptoms mentioned above.
- Require Renal Replacement Therapy.

FACTORS SUGGESTING CHRONICITY[27]:

- Duration of symptoms for months
- Nocturia
- Absence of acute illness in the face of very high urea and creatinine
- Anemia of chronic disorders
- Bone disease
- Sexual dysfunction
- Skin disorders, Nail changes, Pruritus
- Neurological complications
- Small kidneys on renal imaging

PATHOPHYSIOLOGY OF CKD:

Numerous hypothesis have attempted to explain the mechanism underlying progressive CKD. The major ones include

1. Glomerulosclerosis
2. Tubulointerstitial scarring
3. Vascular sclerosis

Glomerulosclerosis:

(a) <u>Hyperperfusion/ Hyperfiltration Hypothesis:</u>

The process of glomerulosclerois occurs at a linear rate in proportion to the reduction in kidney mass leading to an increase in renal plasma flow and hyperfiltration of the remaining nephrons. Glomerular hyperperfusion, hyperfiltration as well as hypertension leads to stretching of the glomerular capillary wall causing epithelial and endothelial injury, transudation of macromolecules into mesangium eventually leading to mesangial overload and dysfunction[28].

The morphological changes of Glomerular Hypertension include

i. glomerular microaneurysms
ii. endothelial detachment
iii. glomerular transudation of proteins
iv. formation of platelet aggregates
v. microthrombi within glomerular capillaries
vi. epithelial stretching
vii. mesangial expansion
viii. foam cell formation

All of the above changes evolve with time to progressive mesangial expansion and sclerosis and ultimately glomerulosclerosis.

(b) Role of Adaptive glomerular hypertrophy:

This change occurs by increased glomerular wall tension, mesangial proliferation and expansion, stretching and damage to glomerular visceral epithelial cells[29].

Glomerular hypertrophy is unlikely to be the sole determinant of glomerulosclerosis. Other factors like Systolic hypertension and hyperlipidemia also contribute.

(c) Role of Systemic Hypertension:

Systemic Hypertension accelerates the progression of chronic nephropathy. In the presence of Hypertension, glomerulosclerosis becomes severe and progressive. The transmission of Systemic Hypertension to the renal glomerular capillary bed is normally prevented by autoregulation of the kidney which determines the severity of glomerular scarring.

When Systemic Hypertension is transmitted to glomeruli, the severity of glomerular scarring depends on the degree of glomerular enlargement and the capillary dilatation. A high Systemic blood pressure at presentation is a cause of poor prognosis and progressive renal failure[30].

(d) Role of Proteinuria:

Proteinuria plays an important role in the initiation of glomerulosclerosis by disrupting the glomerular size and charge permselectivity. Transudation of plasma proteins into the endothelial and subendothelial space occurs leading to glomerular hyalinosis, which is a cause of narrow and occluded glomerular capillaries.

The above said processes lead to increased traffic of macromolecules into the glomerular mesangium and eventually glomerulosclerosis. The presence of proteinuria is a poor prognostic factor in most cases of Chronic progressive renal disorders[31].

(e) Role of Lipids:

Hyperlipidemia leads to glomerular toxixity by causing an increase in the glomerular capillary pressure and functional / structural mesangial changes. Low density lipoprotein - Cholesterol(LDL-C) accumulates in mesangium and matrix. In case of glomerular stress which happens in some inflammatory states, the deposited LDL gets oxidized, taken by the mesangium, forming foam cells. Such modified LDL exerts cytotoxic effects on mesangial, endothelial and epithelial cells[32].

(f) Role of Mediators and Growth factors:

Some mediators and growth factors act on glomerular cells and cause excessive deposition of Extracellular matrix which is not cleared leading to glomerulosclerosis.

Expression in glomerular endothelium:

- Expression of Intercellular Adhesion Molecule-1 (ICAM-1)
- Release of chemotactic and growth promoting mediators
- Release of proaggregatory, proinflammatory, mitogenic cytokines[33] such as IL-1β

Expression in glomerular mesangium:

- Cytoplasmic smooth muscle actin- Migration and contraction of mesangium
- PDGF- Stimulates mesangial proliferation
- TGF-β – Mediates mesangial expansion and sclerosis
- Neutral metalloproteinases and plasmin – related proteases lead to breakdown and turnover of deposited extracellular matrix

Expression in glomerular epithelium:

- Expression of class II antigens of major histocompatability complex
- Release of chemotactic factors- complement components
- Synthesis of PDGF

Tubulo interstitial scarring(TIS):

It correlates better with renal dysfunction and outcome than does glomerular scarring. Severity of interstitial scarring determines the extent of renal dysfunction in patients with diabetic and non-diabetic nephropathies.

(a) <u>Contribution of tubular adaptive changes to the initiation of TIS:</u>

Initiation of TIS can be due to

i. Direct injury

ii. Secondary to glomerular injury

A reduction in functional renal mass leads to both structural and functional adaptive glomerular and proximal convoluted tubular changes.

Functional adaptive changes in turn causes

a. Increase in sodium reabsorption

b. Increase in oxygen consumption and generation

c. Ammoniagenesis by Proximal convoluted tubule[34]

(b) Role of proteinuria:

Proteinuria causes tubular damage by mechanisms such as

1. Excessive reabsorption and overload in proximal convoluted tubule
2. Intraluminal precipitation and obstruction
3. Disruption of tubular membranes with release of Tamm-Horsfall protein into interstitium[35]

(c) Role of lipids:

Lipoproteins are filtered and then reabsorbed by the tubular cells. Lipoproteins are found to be toxic to tubular cells. Balance between oxygen free radicals and anti-oxidants determines the fate of LDL reabsorbed by the proximal convoluted tubule and thus their nephrotoxicity.

Free fatty acids generates a lipid chemotactic factor which initiates tubulointerstitial inflammation by attracting monocytes[36].

(d) Role of Calcium and Phosphate:

Any injury which may be direct or as a consequence of the adaptive changes leads to accumulation of calcium in mitochondria and cytoplasm of tubular cells. Qualitative changes in protein intake and quantitative changes in phosphate intake affect calcification of kidneys[37].

Excessive phosphate in tubular fluid causes precipitation of calcium phosphate leading to tubulointerstitial scarring.

(e) Role of Iron:

In proteinuric states, iron in the tubular lumen causes tubular damage. Free iron in lumen aids in the formation of oxygen free radicals leading to destruction of tubules and acceleration of tubulointerstitial scarring[38].

(f) Role of Mediators and Growth factors[39]:

Tubular cells express adhesion molecules which stimulate interactions between tubular cells and interstitial inflammatory infiltrate and they also express Class II major histocompatabilitycomplex(MHC) which converts proximal convoluted tubule(PCT) to antigen presenting cells.

Tubular cells also release factors like

i. Complement components
ii. Nitric Oxide(NO)
iii. Fatty acid derived Chemotactins
iv. Transforming growth factor-β (TGF-β)
v. Platelet derived growth factor (PDGF)
vi. Tumor necrosis factor-α (TNF-α)
vii. Interleukin-1β (IL-1β)
viii. Epidermal Growth factor (EGF)
ix. Hepatocyte Growth factor (HGF)

Vascular Sclerosis:

Any vascular injury when associated with adventitial expansion causes activation of pericytes into myofibroblasts which migrate to and infiltrate the interstitium of the kidney leading to renal fibrosis[40].

COMPLICATIONS OF CKD[41]:

- Anemia
- Osteodystrophy
- Cardiovascular disease
    - Ischemic heart disease
    - Left ventricular Hypertrophy(LVH)
    - Congestive heart failure
    - Pericarditis
    - Myocardial fibrosis
- Neurological disease
    - Uremic neuropathy
    - Uremic encephalopathy
- Metabolic dysfunction
    - Peripheral Insulin resistance
    - Dyslipoproteinemia
    - Protein malnutrition in patients with End Stage Renal Disease (ESRD) on maintenance dialysis
    - Chronic Kidney Disease – Mineral and Bone Disorder (CKD-MBD)
- Endocrine dysfunction
    - Sexual dysfunction
    - Abnormalities in hormones of hypothalamic-pituitary-gonadal axis

- Gout
- Gastrointestinal Complications[42]
    - Decreased gastric emptying
    - Increased risk of reflux oesophagitis
    - Peptic ulceration
    - Acute pancreatitis
    - Constipation occurs in patients on Continuous Ambulatory Peritoneal dialysis (CAPD)

Hyperphosphatemia in CKD is associated with significant mortality. So a knowledge on phosphate metabolism is absolutely essential.

Phosphate metabolism:

Phosphate (Pi):

Phosphorous (P) is the 6th most common element found in the body . It is important for skeletal mineralization and energy provision processes in the form of ATP [43]. It is an integrated in DNA and RNA, a major constituent of cell membranes and intracellular organelles. It helps in the regulation of intracellular signaling as the substrate for phosphatase and kinase.

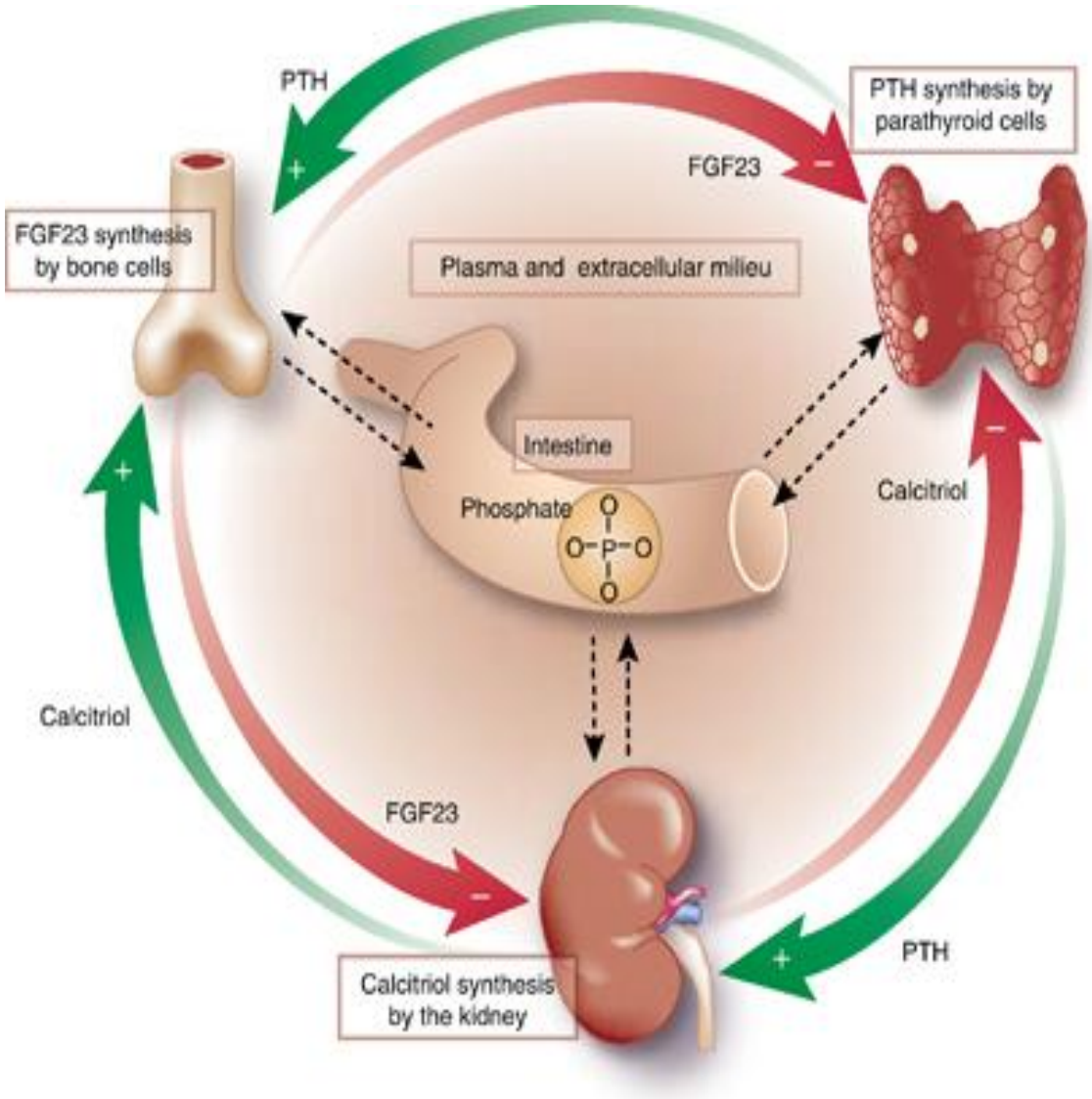
Phosphate Homeostasis :

Phosphorous intake in diet is approximately 1200 mg / day , of which 70% is absorbed from the intestine. Phosphate absorption from intestine is directly proportional to dietary intake (passive absorption) but it is also regulated by active transport through the sodium-dependent phosphate co-transporter type 2 b (NPT2b) to some extent.

Phosphate balance is maintained by the kidney. When phosphate balance is positive, filtered phosphate that is reabsorbed in the proximal convoluted tubules of the kidney falls down leading to phosphaturia and a decrease in serum phosphate levels and vice versa. The reabsorption of phosphate in the kidney is mediated by NPT2a and NPT2c, both of which are expressed in the proximal tubular epithelial cells[44].

Phosphate homeostasis is regulated by extensive hormonal control. Vitamin D regulates the intestinal uptake and Vitamin D together with parathyroid hormone (PTH) regulates the phosphate movement into and out of bone.

Fig.3 Phosphate Homeostasis



PTH decreases the synthesis of NPT2a cotransporters over a long time and also decreases the number of NPT2a cotransporters of the PCT of the kidney on the apical surface. These actions lead to phosphaturia[45]. Hence the main determinant of serum phosphate levels is the expression of NPT2a and NPT2c in the kidney when renal function is normal. Thus an increase in serum phosphate levels is due to renal failure and inability to excrete phosphate load by the kidney. Phosphate homeostasis is deranged in Chronic Kidney Disease – Mineral And Bone Disorder.

CHRONIC KIDNEY DISEASE – MINERAL AND BONE DISORDER (CKD-MBD)[46]

With the progression of CKD, normal systemic mineral homeostasis is not maintained by the kidneys , leading to various abnormalities of bone and mineral metabolism, collectively known as Chronic Kidney Disease – Mineral and Bone Disorder (CKD-MBD).

Definition of CKD-MBD

A systemic disorder of mineral and bone metabolism due to CKD manifested by either one or a combination of the following:

i. Abnormalities of calcium, phosphorous, PTH and / or vitamin D metabolism.
ii. Abnormalities in bone turnover, volume, linear growth, and / or strength.
iii. Vascular and / or other calcifications.

CKD-MBD is a systemic disorder of mineral and bone metabolism which occurs due to CKD, leading to abnormalities in bone and mineral metabolism and / or extraskeletal calcifications. CKD-MBD thus contributes to the progression of kidney injury.

A recently discovered molecule, FGF23, plays a role in the regulation of calcium-phosphate metabolism and thus is also related to the mineral metabolic disorders implicated in CKD-MBD.

FIBROBLAST GROWTH FACTOR 23 (FGF 23):

DISCOVERY[47]:

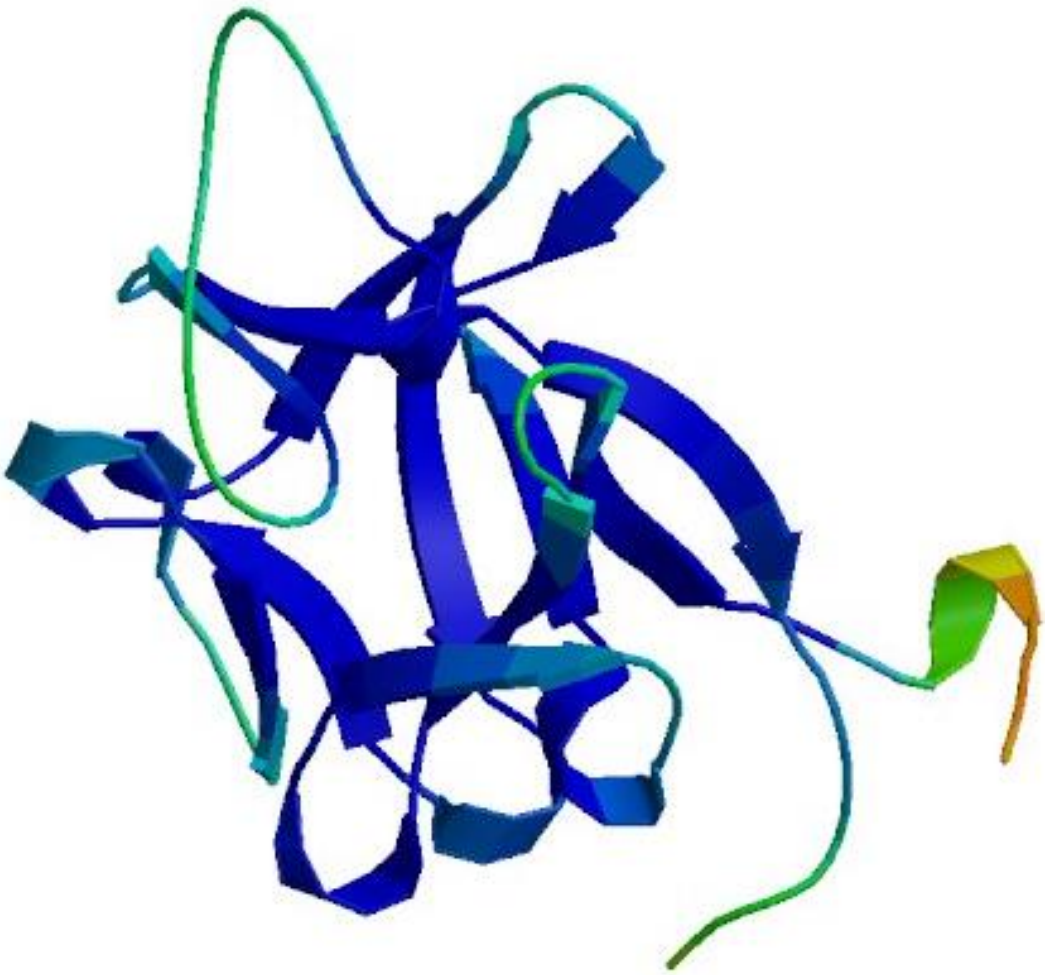
The identification of FGF23 by positional cloning in patients with Autosomal Dominant Hyperphosphatemic rickets(ADHR) showed that FGF23 gene is responsible for inducing phosphate wasting by inhibiting the Sodium / Phosphate (Na/Pi) cotransporter system in kidney.

Using the mouse FGF23 sequence, Yamashita et al. (2000) identified FGF23 in a genomic database. They cloned the full-length cDNA from a placenta library. The deduced 251-amino acid protein contains an N-terminal 24-amino acid signal sequence. FGF23 shares 72% sequence identity with mouse FGF23, and 24% and 22% identity with human FGF21 and FGF19, respectively. By quantitative PCR, it has been found highest expression of FGF23 in brain and lower expression in thymus[48].

MOLECULAR GENETICS[49]:

FGF23 is a 26 KDa protein with about 251 aminoacid residues secreted by the osteoblasts. The ADHR Consortium (2000) found that the FGF23 gene

Fig.4 Structure of Human FGF23 molecule



lies 54kb telomeric of FGF6 on 12p13. It is composed of 3 exons spanning 10kb of genomic sequence.

It was identified that 3 missense mutations occurred in the FGF23 in affected members of 4 unrelated families with autosomal dominant hypophosphatemic rickets . These mutations, which represented the first found in a human FGF gene causing disease, affected 2 arginines that lie only 3 amino acids apart. This finding showed that the ADHR phenotype is caused by a gain-of-function mechanism.

CHARACTERISTICS OF FGF23:

Fibroblast growth factors (FGFs) include a polypeptide family that have 120 highly conserved amino acid residues in the common core region and a variable flanking N- and C-terminal aminoacid residues. FGF23 contains a N-terminal signal peptide, an FGF-like sequence and a C-terminal extended sequence[50].

FGF23 has to be O-glycosylated at the threonine residue at position 178 for its transportation from the Golgi apparatus through the cytoplasm to be finally secreted. Polypeptide N-acetyl galactosaminyl transferase 3 (GALNT3) is the enzyme responsible for the post-translational modification of FGF23. GALNT3 mutations cause familial tumoral calcinosis and hyperphosphatemia due to a post-translational processing defect in FGF23.

Seven subfamilies of human FGFs are known. FGF19, FGF21 and FGF23 are members of the FGF19 subfamily[51]. The FGF19 subfamily members' affinity for heparin is low, and so they are distributed throughout the body, through bloodstream, to mediate their systemic effects. The half-life (t½) of intact FGF23 in the circulation of normal individuals is approximately about 58 minutes[52].

PHYSIOLOGY OF FGF23:

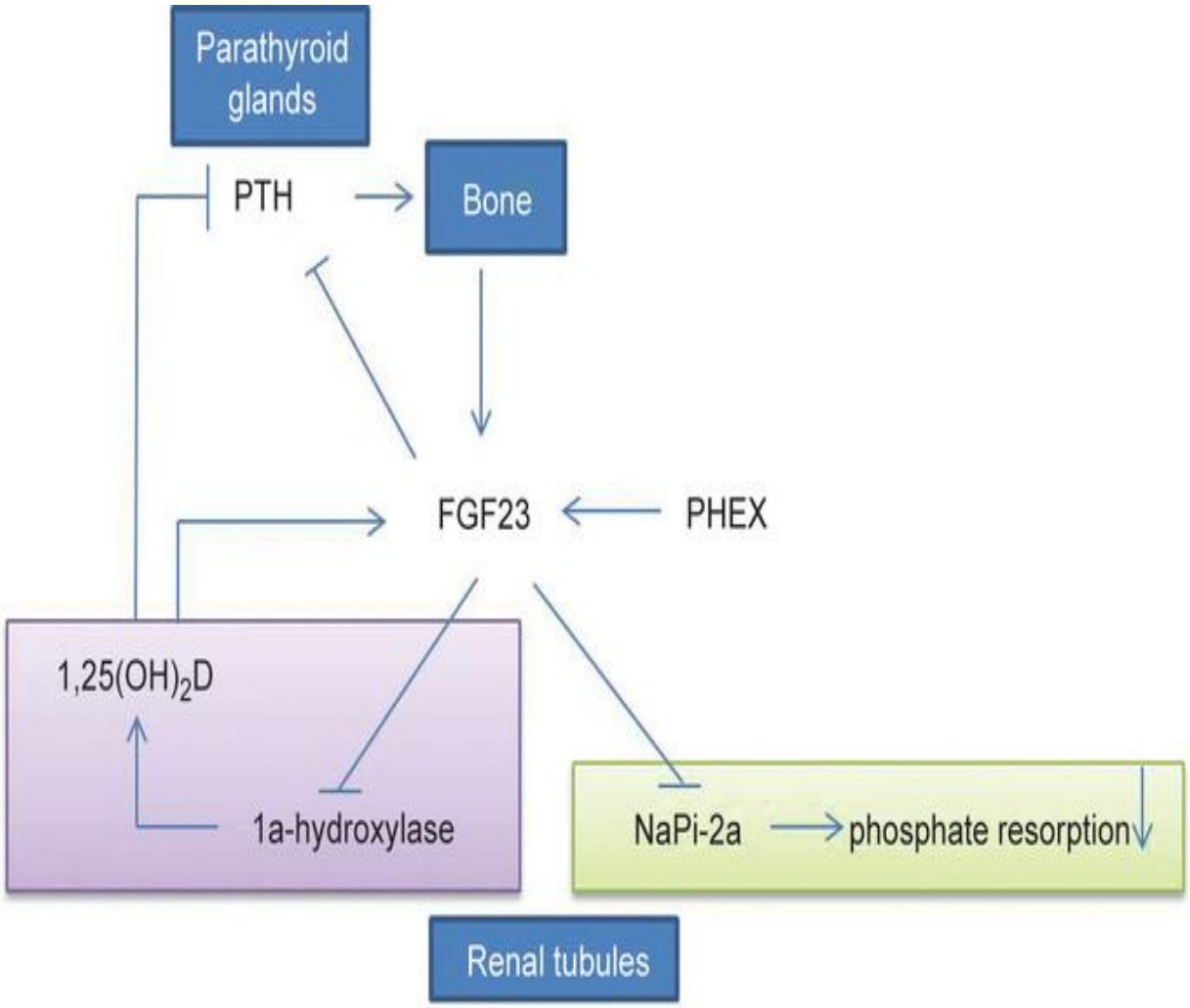
FGF23 functions as a hormone to act on specific target organs and regulates diverse metabolic processes. It is expressed mainly in bone (primarily by osteoblasts and osteocytes). FGF23 acts on its receptor complex, Klotho-FGFR1(FGF Receptor-1) in the kidney to cause phosphaturia and to decrease the synthesis of calcitriol[53].

Klotho is a co-receptor for FGF23. It is a 130 KDa transmembrane β-glucuronidase causing hydrolysis of steroid β-glucuronides. Klotho is expressed only in limited tissues, mainly the kidneys, parathyroid glands and the choroid plexus.

BIOLOGICAL ACTIONS OF FGF23:

1. The full-length intact FGF23 is a phosphaturic hormone. FGF23, by decreasing the expression of the sodium-phosphate co-transporters NPT2a and NPT2c in the proximal tubule of the kidney causes phosphaturia. This process occurs through mitogen-activated protein kinase (MAPK) pathway[54].
2. FGF23 causes decreased conversion of 25- Hydroxy vitamin D (25(OH)D) to its active metabolite $1,25(OH)_2D$ by suppressing renal 1-alpha hydroxylase (1α hydroxylase). FGF23 by stimulating 24- hydroxylase , also causes reduction of 25(OH)D and $1,25(OH)_2D$ levels, since 24- hydroxylase causes vitamin D degradation[55].
3. In the parathyroid gland, FGF23 causes a reduction in PTH expression and secretion, and increases 1α-hyroxylase mRNA levels, which contrasts with the negative effects of FGF23 on 1α-hydroxylase expression in the kidney[56].

Fig.5 Biological Actions of FGF23



REGULATION OF FGF23:

Several factors have a direct or indirect influence on serum FGF23 levels.

Phosphate

FGF23 levels rise in response to phosphate loading. In humans, dietary phosphorus affects serum FGF23 levels. In patients with chronic hypoparathyroidism, serum FGF23 levels increase in response to increased serum phosphate levels. In healthy individuals, acute increase and decrease of serum phosphate levels do not cause any alteration in circulatory FGF23 levels, which reveals that, in the rapid adaptation of phosphate homeostasis, FGF23 is not involved.

FGF23 levels are affected by dietary phosphorous load, whereas non-dietary interventions such as intravenous phosphorous infusion do not change FGF23 levels[57].

Vitamin D

Calcitriol, mediating through a vitamin D responsive element (VDRE) in the FGF23 promoter stimulates the production of FGF23 in bone and osteoblasts independent of serum phosphate and PTH[58].

PTH

In humans, the effect of PTH on circulating FGF23 is that it directly modifies serum phosphate levels which then leads to altered serum FGF23 levels and is under investigation[59].

Estrogen

Estrogens cause increased transcription and translation of FGF23 in osteoblasts, in a concentration and time dependent manner in some in-vitro studies[60].

Genetic factors

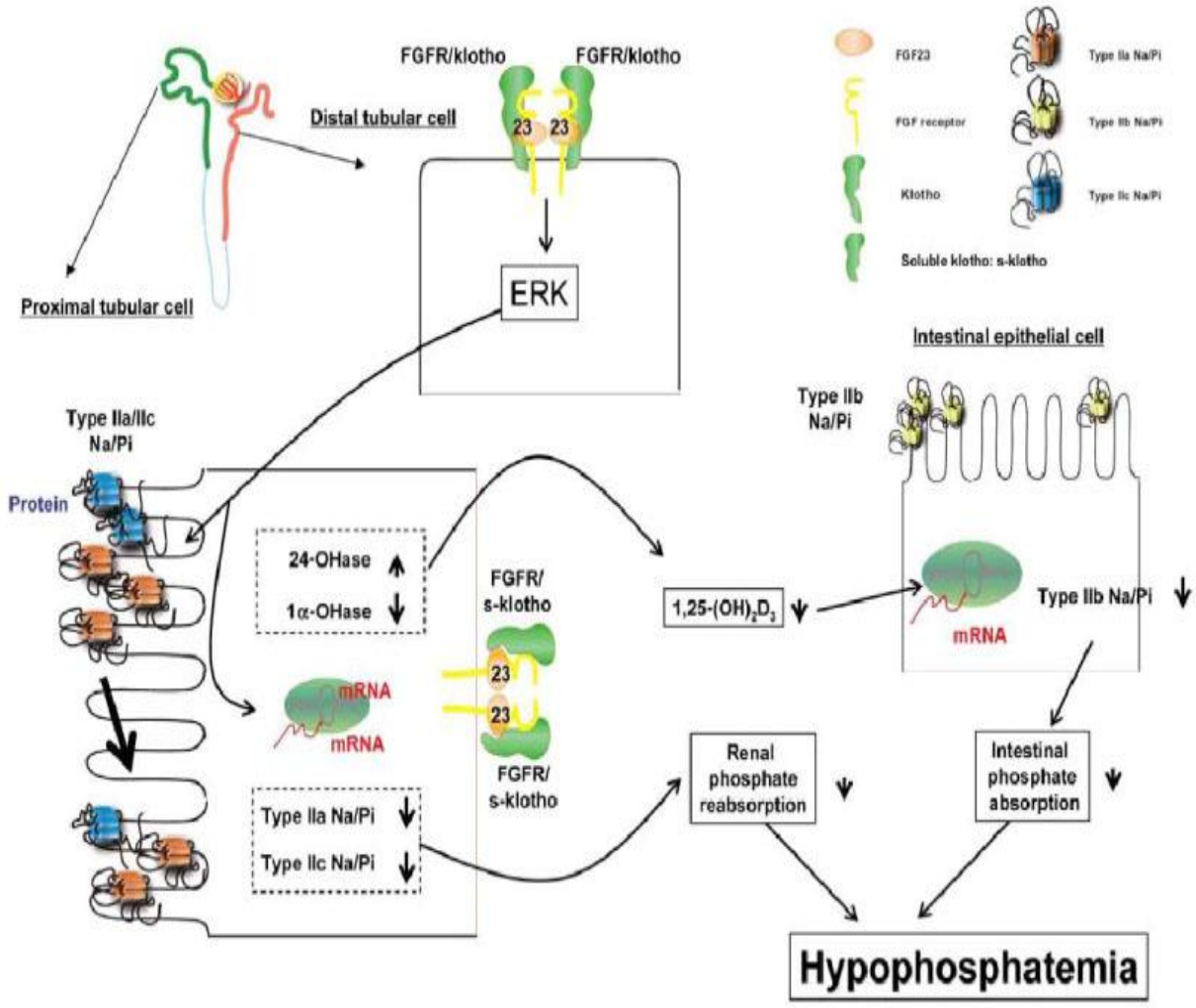
Genetic defects can result in overstimulation of serum FGF23 levels as in ADHR and X-linked Hypophosphatemic rickets(XLH) either by direct or indirect action. XLH is caused by inactivating mutations of PHEX, a phosphate-regulating gene with homologies to endopeptidases on the X chromosome[61].

FIBROBLAST GROWTH FACTOR RECEPTORS(FGFRs) & FGF23 SIGNALING:

FGF23 signaling was not very well understood before the discovery of alpha-Klotho . FGF23 exerts its actions mainly through activation of FGFRs in a type I membrane-bound Klotho dependent manner. This is because Klotho binds directly to multiple FGFRs forming a Klotho-FGFR complex. This complex has a higher affinity binding to FGF23 than does FGFR or Klotho alone.

Klotho signaling improves the ability of FGF23 to induce ERK and FGF receptor substrate phosphorylation in many cell types. The tissue distribution of Klotho determines the target specificity of the FGF19 subfamily members[62].

Fig.6 Mechanism of FGF23



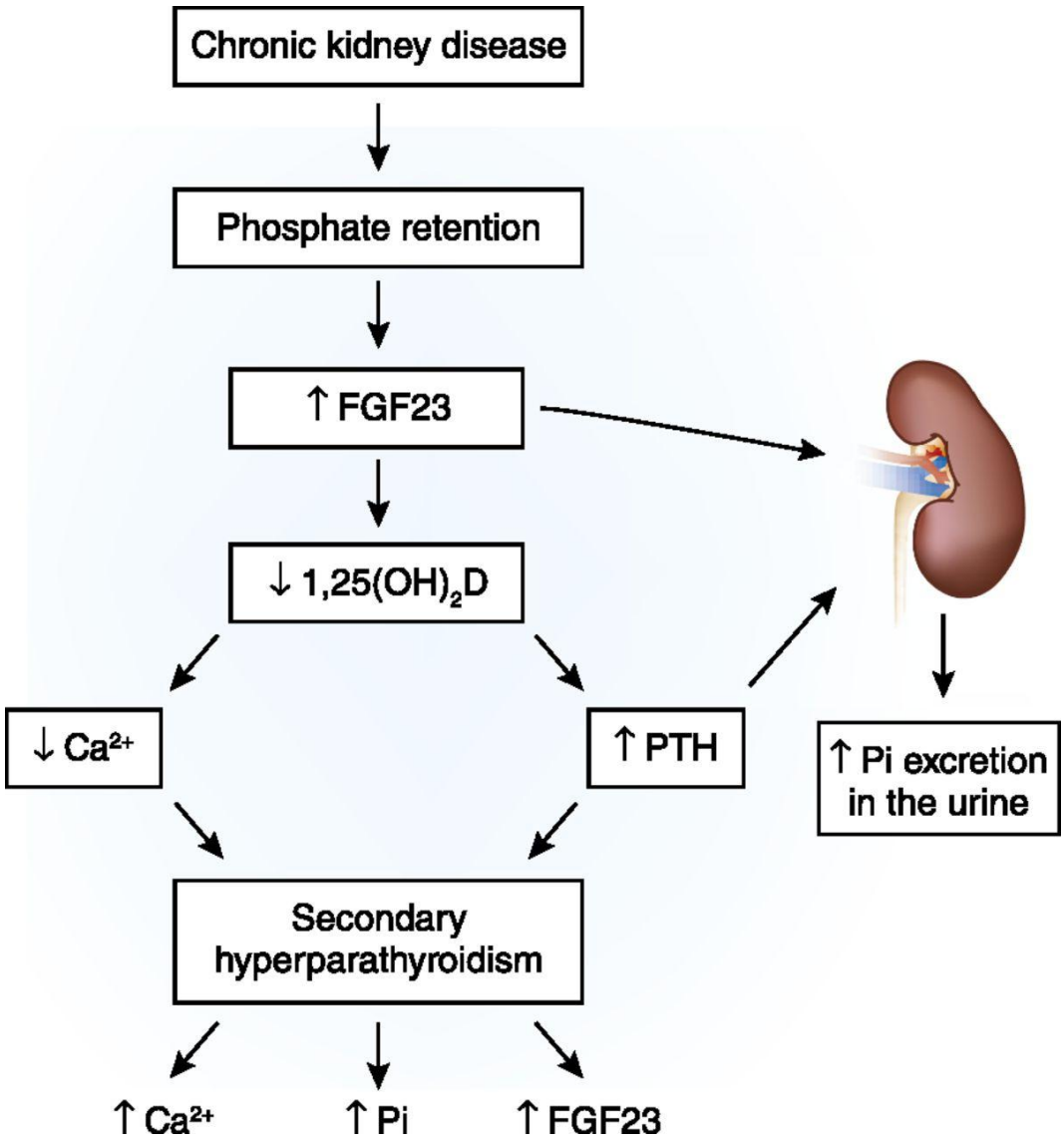
ROLE OF FGF23 IN CKD:

In CKD, since the failing kidney is not able to adequately maintain mineral homeostasis, it initiates a series of events that lead to biochemical changes in serum, altered bone metabolism, vascular calcification and increased morbidity and mortality.

FGF23 levels increase in early stages of CKD and the levels rise progressively with a decline in GFR[63]. During the initial stages of CKD, despite a declining nephron mass, serum phosphate levels are normally maintained, due to progressively increasing FGF23 levels. FGF23 stimulates excretion of phosphate through the remaining nephrons and it also inhibits $1,25(OH)_2D$ synthesis thereby decreasing the absorption of dietary phosphorous[64].

Hyperphosphatemia is observed only with advanced renal disease (GFR<30 mL/min/1.73 sq.m), while serum FGF23 levels increase earlier in CKD[65,66]. In ESRD, FGF23 levels are so much elevated and those patients undergoing hemodialysis have upto 1000-fold serum FGF23 levels above the normal range[67].

Fig.7 Role of FGF23 in CKD



Two hypothesis have been proposed to explain the increase in FGF23 concentrations early in the course of CKD which include

1. Kidney injury induces the production of an unknown factor that stimulates FGF23 secretion.
2. In low Klotho expression states, higher FGF23 may be required to overcome Klotho deficiency[68].

Elevated serum phosphate, high PTH and low calcitriol levels are each independently associated with cardiovascular morbidity and mortality in CKD[69]. The more recently discovered molecule FGF23, when increased is more significantly and independently associated with mortality and progression of CKD.

It is of particular interest to analyze the role of FGF23 in phosphate homeostasis in healthy individuals and in those with CKD since FGF23 is a potent regulator of both phosphate and vitamin D and it is also a very early marker of CKD-MBD.

AIMS AND OBJECTIVES

AIMS AND OBJECTIVES

AIM:

To estimate the levels of serum Fibroblast Growth Factor 23 (FGF23) in patients with CKD and to compare them with healthy controls.

OBJECTIVES:

1. To evaluate the role of FGF23 as an early marker for CKD – MBD.
2. To estimate serum FGF-23 levels in chronic kidney disease patients and healthy control (age and sex matched) groups.
3. To compare the levels of FGF-23 levels with creatinine clearance and kidney size in various stages of CKD.

MATERIALS AND METHODS

MATERIALS AND METHODS

The study was cross-sectional in design and it was conducted at Mahatma Gandhi Memorial Government Hospital attached to K.A.P.V Government Medical College, Trichy during the period of June 2014- May 2015.

STUDY POPULATION:

45 patients with CKD was considered as cases and 45 healthy volunteers was considered as controls.

INCLUSION CRITERIA:

1. Patients with established diagnosis of CKD
2. Age between 20 – 75 years.

EXCLUSION CRITERIA:

1. Acute / chronic inflammatory diseases (sepsis, infection, malignancy and liver disease)
2. Previous history of Coronary Artery Bypass Graft surgery
3. Acute kidney injury
4. Patients on immunotherapy
5. Previous history of cerebrovascular diseases
6. Patients who underwent renal transplantation

Informed consent was obtained from all subjects prior to the study. Under aseptic precautions, 5ml of venous blood sample was collected after an

overnight fasting of 12 hours from all subjects. After retraction of the clot, samples were centrifuged at 2000rpm for 15 minutes for separation of serum.

An aliquot of the serum was taken for the estimation of FGF23 and stored at -20°C in the deep freezer. The remaining serum was used for the estimation of Glucose, Urea, Creatinine, Electrolytes (Sodium, Potassium), Albumin, Calcium, Phosphorus, Alkaline Phosphatase.

Spot Urine sample was collected to determine the presence of albumin. Ultrasound Abdomen was done to determine the kidney size and cortico-medullary differentiation.

ANALYSIS OF SAMPLES :

ESTIMATED PARAMETERS :

1. Serum FGF-23	- Enzyme-linked immunosorbent assay (ELISA)
2. Fasting Blood Glucose (FBG)	- Glucose oxidase and peroxidase method
3. Blood Urea	- Urease - Glutamate Dehydrogenase (GLDH) method
4. Serum Creatinine	- Modified Jaffe's kinetic method
5. Serum Uric acid	- Uricase method
6. Serum Electrolytes (Sodium , Potassium)	- ISE method
7. Serum Albumin	- BCG dye binding method
8. Serum Calcium	- Arsenazo method

9. Serum Phosphorus - Ammonium Molybdate Kinetic method

10. Serum Alkaline Phosphatase - DEA-PNPP Kinetic method

11. Urine Albumin - Dipstick method

CALCULATED PARAMETER:

1. eGFR – Chronic Kidney Disease-Epidemiological(CKD-EPI) Formula

OTHER INVESTIGATION:

2. Ultrasound Abdomen

ESTIMATION OF SERUM FIBROBLAST GROWTH FACTOR 23 (FGF23):

Method:

Enzyme Immuno Assay

Principle:

The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of Human fibroblast growth factor 23(FGF23) in samples. Human FGF-23monoclonal antibody is precoated onto a micro-titer plate. After a blocking step and incubation of the plate with anti-FGF23 antibody, Biotinylated FGF23 and standard FGF23 (or) samples are added to all wells. There is a competitive binding between biotinylated FGF23 and standard (or) serum FGF23 with anti- FGF23 antibody. Streptavidin–Horseradish Peroxidase (HRP) was added to the well which reacts with the uncompleted (or) free biotinylated FGF23 to produce a colour. The Intensity of the colour is directly proportional to the amount of biotinylated FGF23 and inversely proportional to the amount of FGF23 peptide in the standard or

sample. The concentration of FGF23 in the serum is calculated from a standard curve of different FGF23 concentrations accordingly.

Reagents:

1. Standard (1600pg/ml) - 0.5ml
2. Standard diluent - 3ml
3. Micro-ELISA Strip plate - 12w×8s
4. Streptavidin- HRP-Conjugate Reagent - 6ml
5. 30×wash solution - 20ml
6. Biotin-FGF-23 Ab - 1ml
7. Chromogen Solution A - 6ml
8. Chromogen Solution B - 6ml
9. Stop Solution - 6ml

Reagent Preparation:

1. Kit reagents were kept on ice during reagent preparation.
2. Preparation of Standards:

   7 micro-tubes were labeled with the following concentrations:

Tube no 7	1600pg/ml
Tube no 6	800pg/ml
Tube no 5	400pg/ml
Tube no 4	200pg/ml
Tube no 3	100pg/ml
Tube no 2	50pg/ml
Tube no 1	0pg/ml

The original FGF23 stock standard supplied by the kit manufacturer had 0.5ml of concentration 1600pg/ml. From this stock standard, a series of working standards were prepared by mixing it with standard diluents. From the stock standard, 120µl was added to the tube 6 labeled as 800pg/ml and 120 µl of standard diluent was added which served as the working FGF23 standard solution of concentration 800ng/ml. This step was repeated after thorough mixing before each transfer with each successive concentration, except the last tube preparing a dilution series as shown in the following table and illustration.

STANDARD CONCENTRATION	**STANDARD**	**TUBE NO.**	**DILUTION**
1600pg/ml	Standard No.7	7	120µl Original Standard
800pg/ml	Standard No.6	6	120µl Original Standard + 120µl Standard diluent
400pg/ml	Standard No.5	5	120µl Standard No.6 + 120µl Standard diluent
200pg/ml	Standard No.4	4	120µl Standard No.5 + 120µl Standard diluent
100pg/ml	Standard No.3	3	120µl Standard No.4 + 120µl Standard diluent
50pg/ml	Standard No.2	2	120µl Standard No.3+120µl Standard diluent
0pg/ml	Standard No.1	1	----

The final tube served as the Zero standard, the concentration of which is 0pg/ml of FGF23.

1600pg/ml 800pg/ml 400pg/ml 200pg/ml 100pg/ml 50pg/ml

Wash buffer Preparations:

30X wash concentrate was brought to room temperature . To 20 ml of the wash solution, 880 ml of distilled water was added to yield 900ml of wash buffer.

Assay Procedure:

1. Micro-titre plate was equilibrated to room temperature before opening the sealed pouch.
2. To the standard wells, 50µl of standard and 50µl of Streptavidin-HRP was added and since the standard already had combined biotin antibody, it was not added separately.
3. To the test wells, 40µl of sample was addedand then both 10µl of FGF23-antibody and 50µl of Streptavidin-HRP was added.
4. Then seal the sealing membrane , and gently shaking, incubated 60 minutes at 37ºC.
5. The membrane was removed carefully and the liquid was drained.

6. Plate was washed five times with wash buffer.
7. 50µl of chromogen solution A and 50µl of chromogen solution B was added to each well, gently mixed, incubated for 10 min at 37ºC away from light.
8. 50µlof Stop Solution was added into each well to stop the reaction (the blue turned into yellow immediately).
9. The optical density (OD) was measured at 450 nm wavelength within 15 min of adding the stop solution.

Calibration Graph:

By plotting the mean absorbance of each standard on the y-axis against the concentration of FGF23 (pg/ml) in each standard on the x-axis, a calibration curve was constructed.

Sensitivity:

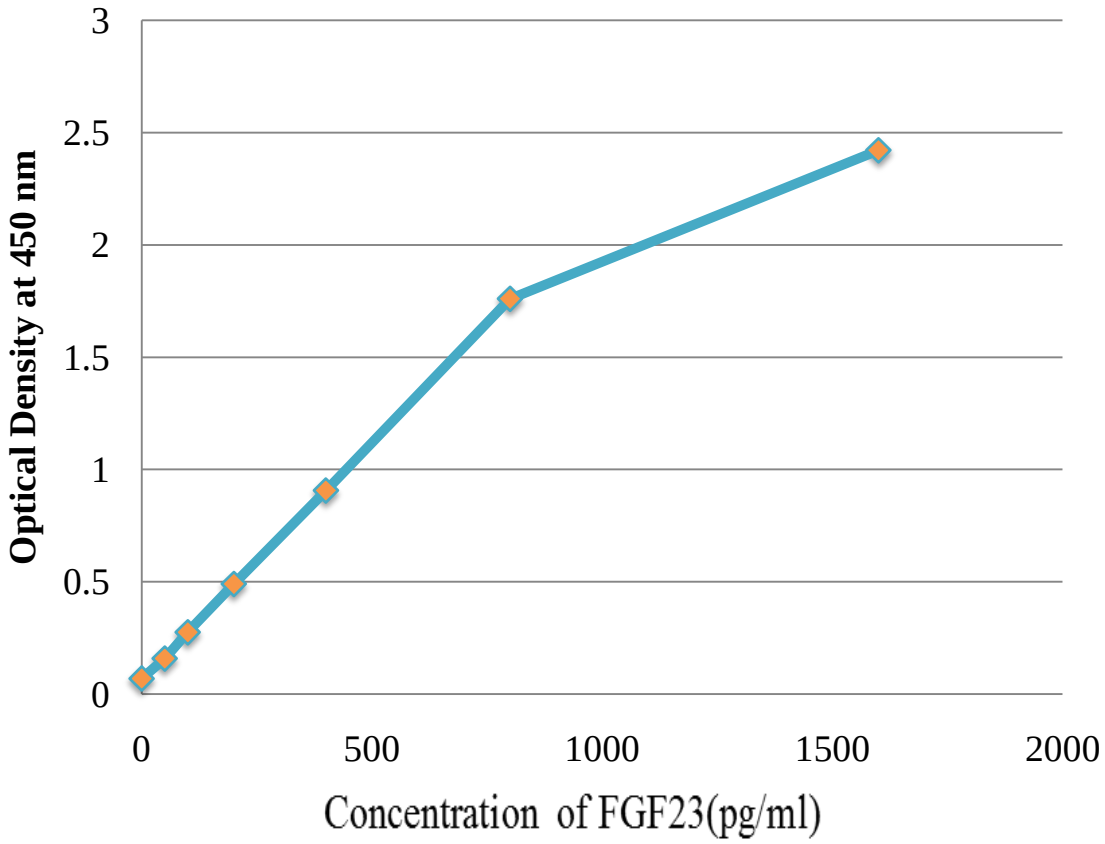
The minimum detectable concentration of Serum FGF23 is 2.49 pg/ml .

Linearity:

The linearity of serum FGF23 lies between 5 - 1500 pg /ml.

Reference range of Serum FGF23 : 8.2 – 54.3pg /ml.

CALIBRATION GRAPH OF FGF23 STANDARDS



Estimation of Glucose:

Method : Glucose oxidase – peroxidase (GOD-POD) method.

Analysis : End Point Analysis

Principle :

Glucose is oxidized to yield gluconic acid and hydrogen peroxide in the presence of glucose oxidase. Hydrogen peroxide oxidatively couples with 4-amino antipyrine and phenol to produce red quinoneimine dye in the presence of peroxidase. This red dye has maximum absorbance at 505nm. The intensity of the coloured complex is directly proportional to the concentration of glucose in specimen.

D-Glucose + O_2 + H_2O $\xrightarrow{GOD}$ Gluconic acid + H_2O_2

H_2O_2 + 4- amino antipyrine + Phenol $\xrightarrow{POD}$ red dye + H_2O

Specimen: Fresh unhemolysed serum

Assay Procedure:

Enzyme reagent and standard were brought to the room temperature before performing the assay.

Reagents	**Blank**	**Standard**	**Sample**
Glucose enzyme reagent	1000µl	1000µl	1000µl
Standard	-	10µl	-
Sample	-	-	10µl
Distilled water	10µl	-	-

The tubes were mixed thoroughly and incubated at 37°C for 10 min. The absorbance was read against reagent blank at 505nm.

Calculation:

Glucose (mg/dl) = $\frac{\text{Absorbance of test}}{\text{Absorbance of standard}}$ x Concentration of standard (mg/dl)

Glucose Standard	:	100mg/dl
Linearity	:	Upto 500mg/dl
Normal Values	:	90-120mg/dl
Glucose fasting	:	60-100mg/dl
Glucose postprandial	:	90-140mg/dl

ESTIMATION OF UREA

Method:

Urease – GLDH method

Principle:

Urea is hydrolyzed to ammonia and carbon dioxide by Urease. Then Glutamate dehydrogenase (GLDH) converts Ammonia and α- Ketoglutarate to Glutamate and water with the concurrent oxidation of NADH to NAD^+ . Two moles of NADH are oxidized for each mole of urea present.

$$\text{Urea} + H_2O \xrightarrow{Urease} 2NH_4^+ + CO_2.$$

$$NH_4^+ + \text{NADH} + H^+ + \text{2-oxoglutarate} \xrightarrow{GLDH} \text{Glutamate} + NAD^+.$$

The initial rate of decrease in absorbance at 340nm is proportional to the urea concentration in the sample.

Reagent composition:

Reagent 1:

α-KetoGlutaric Acid 99.8mmol/L

Urease 23.5kU/L, GLDH 3.5KU/L, Adenosine diphosphate 7.6mmol/L, Sodium azide 0.2%.

Reagent 2:

NADH 2.95mmol/L, sodium azide 0.1%

Concentration of Urea standard - 50mg/dl.

Reagent Preparation:

Working reagent was prepared by mixing 4 parts of reagent 1 with one part of reagent 2.

Procedure:

3 test tube were labeled as Blank, standard and test and the procedure is done as follows:

Tubes	Working reagent	Standard	Test sample	Distilled water
Blank	1000µl	-	-	10µl
Standard	1000µl	10µl	-	-
Test	1000µl	-	10µl	-

The tubes were mixed well and the absorbance was read after 20 seconds (A_1) and 60 sec (A_2) at 340nm.

Calculation:

$\Delta A = A_2 - A_1$.

Urea in mg/dl = $\frac{\Delta A \text{ of test}}{\Delta A \text{ of standard}}$ × Concentration of standard

Linearity:

The method is linear upto 200mg/dl.

Reference Interval:

Adults 15-30mg/dl

ESTIMATION OF SERUM CREATININE

Method: Modified Jaffe's Method.

Principle:

Creatinine reacts with alkaline picrate to produce an orange-yellow colour. The intensity of the colour is directly proportional to the concentration of Creatinine and is measured photometrically at 510nm.

Concentration of creatinine standard - 2 mg/dl

Reagent Composition:

Reagent No	Composition	Concentration
1	Picric acid	25.8mmol/L
2	Sodium hydroxide	95mmol/L

Reagent Preparation:

Equal Volumes of reagent 1 and reagent 2 were mixed and waited for 15 minutes before use.

Procedure:

3 test tubes were taken and labeled as Blank, Standard and test and the procedure was done as follows:

Tubes	**Working reagent**	**Standard**	**Test sample**	**Distilled water**
Blank	1000µl	-	-	100µl
Standard	1000µl	100µl	-	-
Test	1000µl	-	100µl	-

The tubes were mixed well and the absorbance was read after 20 seconds (A_1) and 80 sec (A_2) at 510nm, against reagent blank with distilled water.

Calculation:

$\Delta A = A_2 - A_1$.

$$\text{Serum Creatinine (mg/dl)} = \frac{\Delta A \text{ of test}}{\Delta A \text{ of standard}} \times \text{Concentration of standard}$$

Linearity:

This assay is linear upto 20mg/dl.

Reference Range for serum creatinine:

Males : 0.7-1.4 mg/dl

Females : 0.6-1.2mg/dl

ESTIMATION OF URIC ACID:

Method: Uricase method

Principle:

Uric acid is acted on by uricase forming allantoin and hydrogen peroxide. The hydrogen peroxide reacts with phenolic chromogens in presence of peroxidase forming a red coloured compound.

$$H_2O_2 + \text{Phenolic chromogen} \xrightarrow{\text{Peroxidase}} \text{Red coloured compound}$$

This compound has maximum absorption at 520 (500 – 530) nm. Absorption is directly proportional to the concentration of uric acid.

Procedure:

Reagent	**Sample**	**Standard**	**Blank**
Working reagent	1ml	1ml	1ml
Sample	25 µl	-- --	---
Standard	---	25 µl	---

Incubate for 10 minutes at room temperature. After completion of incubation , measure absorbance of acyl mixture against blank at 520nm.

Concentration of Uric acid Standard – 6mg/dl

Calculation:

Amount of uric acid present in 100 ml of plasma or serum

$$= \frac{\text{OD test} - \text{OD blank}}{\text{OD std - OD blank}} \text{ X } \frac{\text{Conc. Of std}}{\text{Volume of sample}} \text{ X } 100$$

$$= \frac{\text{OD test} - \text{OD blank}}{\text{OD std - OD blank}} \text{ X } \frac{0.0015}{0.025} \text{ X } 100 \text{ mg/dl}$$

$$= \frac{\text{OD (T)} - \text{OD (B)}}{\text{OD (S) - OD (B)}} \text{X } 6 \text{ mg/dl}$$

Result: The concentration of uric acid in given sample of serum = -------- mg%

Reference Range of Uric acid :

Males : 3.5 to 7 mgs %

Females : 2.5 to 6 mgs %

ESTIMATION OF SERUM ELECTROLYTES (Sodium/Potassium):

Method : Ion selective Electrode

Analysis time : 60 seconds

Detection Range : 20-200 mEq/L

Reference Range : Serum Sodium : 135 – 145 mEq/L

Serum Potassium : 3.5 – 5 mEq/L

ESTIMATION OF SERUM ALBUMIN :

Method : Dye (Bromocresol Green) Binding Method.

Principle:

At pH 3.68, albumin acts as a cation and binds to the anionic dye BromocresolGreen(BCG) forming a green coloured complex. The absorbance is measured at 630 nm. The colour intensity of the complex is proportional to albumin concentration in the sample.

$$\text{Albumin} + \text{BCG} \xrightarrow{\text{pH 3.68}} \text{Green coloured complex}$$

Procedure:

	Blank	**Standard**	**Test**
Reagent	1 ml	1 ml	1 ml
Standard	-	10 µl	-
Sample	-	-	10 µl

Mix well, incubate at room temperature for 1 minute and read at 630 nm.

Concentration of standard – 4g/dl

Calculation:

Amount of albumin present in 100 ml of plasma or serum

$$= \frac{OD\ (T) - OD\ (B)}{OD\ (S) - OD\ (B)} \times \frac{\text{Conc. of std}}{\text{Volume of sample}} \times 100$$

$$= \frac{OD\ (T) - OD\ (B)}{OD\ (S) - OD\ (B)} \times \frac{0.0004}{0.01} \times 100$$

$$= \frac{OD\ (T) - OD\ (B)}{OD\ (S) - OD\ (B)} \times 4\ g/dl$$

Result: The concentration of albumin in given sample of serum = -------- g%

Reference range for Serum Albumin:

Adult: 3.5 – 5.2 g/dl

ESTIMATION OF CALCIUM:

Method : Arsenazo III Method

Principle : Calcium with Arsenazo III (1,8-Dihydroxy-3,6-disulpho-2,7-naphthalene-bis(azo)-dibenzenearsonic acid) , at neutral pH, yields a blue coloured complex. The intensity of the colour formed is directly proportional to the calcium concentration in the sample.

Reagent Composition:

Reagent I – Arsenazo III Reagent

Calcium Standard – 10mg/dl

Procedure:

	Blank	**Standard**	**Sample**
Sample	-----	-----	25µl
Standard	-----	25µl	-----
Reagent	1000 µl	1000 µl	1000 µl

The tubes were mixed well, incubated at room temperature for 5 minutes. The final absorbance of the sample (A_c) and standard (A_s) were measured against the reagent blank.

Calculation:

Concentration of Calcium in Serum =

$$\frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \text{ X Concentration of standard (mg/dl)}$$

$$= (A_c / A_s) \times 10$$

Result: The concentration of serum calcium in the given sample = ------ mg%

Linearity:

The method is linear upto a concentration of 16mg/dl

Reference Range for Serum Calcium: 8.8 – 10.2 mg/dl

ESTIMATION OF SERUM PHOSPHORUS:

Method : Ammonium Molybdate Kinetic Method

Principle : Inorganic phosphate reacts with ammonium molybdate in presence of sulphuric acid to give ammonium phosphomolybdate. The formation of phospho - molybdate is measured at 340 nm and it is directly proportional to the concentration of inorganic phosphorus present in the sample.

Inorganic phosphate + Ammonium molybdate $\longrightarrow$ Ammonium phosphomolybdate

Reagent Composition:

Reagent 1: Phosphorus Reagent

Ammonium molybdate	0.43 mmol/L
Sulphuric acid	213 mmol/L
Surfactant	-------

Concentration of Phosphorus Standard – 5 mg/dl

Procedure:

Reagent	Blank	Standard	Test
Molybdate reagent	1ml	1ml	1ml
Standard	---	10 µL	--
Sample	---	---	10µ L

Mix well and incubate for 5minute measure the absorbance of sample, standard against reagent at 340nm.

Calculation:

Phosphorus (mg/dl) =

$$\frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \text{ X Concentration of standard (mg/dl)}$$

Result:

The concentration of inorganic phosphorus in the given sample = -------- mg%

Reference range for Serum Phosphorus :

Adult : 2.5 – 4.5 mg/dl

ESTIMATION OF SERUM ALKALINE PHOSPHATASE

Method : Diethanolamine- para-nitro phenyl phosphate (DEA-PNPP) , Kinetic

Principle:

Alkaline Phosphatase (ALP) at an alkaline pH hydrolyses para-nitro phenyl phosphate into para-nitro phenol and inorganic phosphates. The rate of formation of para-nitro phenol is measured at 405nm as an increase in absorbance which is directly proportional to alkaline phosphatase activity in the sample.

$$\text{p - nitrophenyl phosphate} + H_2O \xrightarrow{\text{ALP}} \text{p-nitro phenol} + \text{Inorganic hosphates}$$

Reagent Composition:

R1- DEA Buffer Reagent

R2- PNPP Reagent

Reagent Preparation

R1 and R2 were mixed in the following proportion

R1- DEA Buffer Reagent – 800 µl

R2- PNPP Reagent – 200 µl

Procedure:

Reagent	**Blank**	**Test**
Working reagent	1ml	1ml
Standard	---	---
Sample	---	20 µl

The sample and the reagent are mixed well and the initial absorbance (A_0) is read at 405nm and repeated absorbance readings are taken after every 1,2& 3 minutes for total 3 minutes time. The mean absorbance change per minute ($\Delta A/min$) is calculated.

Calculation:

ALP activity in U/L = ($\Delta A/min$) x 2750

Result:

The concentration of serum alkaline phosphatase in the given sample = --- U/L

Linearity: The kit is linear upto 2000 U/L

Reference Range for Serum Alkaline Phosphatase:

Males : 80 – 306 U/L

Females : 64 – 306 U/L

ESTIMATION OF URINE ALBUMIN

Method: Dipstick Method

Principle :

This is based on the protein-error-of indicator principle. The reagent area is more sensitive to albumin than other proteins. Anion in the specific pH indicator attracted by cation on protein molecule makes the indicator further ionise, which changes its colour.

Reactive Ingredients:

Tetrabromphenol blue	0.1% w/w
Buffer	97.4% w/w
Non reactive ingredients	2.5% w/w

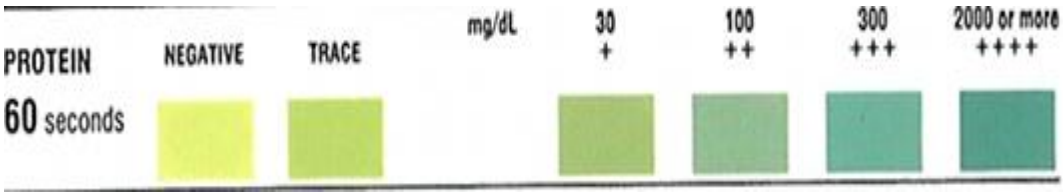
Collection and Preparation of Specimen:

Fresh urine is to be collected in a clean and dry container. Centrifuging of urine should not be done. The sample should be mixed well before testing. The urine testing has to be done within an hour of collection.

Visual Reading Technique:

Immerse the reagent area of the strip in the urine specimen and take it out immediately. Run the edge of the strip against the rim of the container to remove the excess urine. Hold up the strip up horizontally and compare the result on the strip with the colour chart on the bottle label closely. Make note of the result. For semi-quantitative results of protein, the results are read within 60 seconds after dipping.

Protein dipstick grading		
Designation	**Approx. amount**	
	Concentration	**Daily**
Trace	5–20 mg/dL	
1+	30 mg/dL	Less than 0.5 g/day
2+	100 mg/dL	0.5–1 g/day
3+	300 mg/dL	1–2 g/day
4+	More than 300 mg/dL	More than 2 g/day



Sensitivity and Test range of Urinanalysis strips:

Item	Sensitivity	Visual Test range
Protein(g/L)	0.15-0.3	Neg.- 20.0

CALCULATED PARAMETER:

eGFR calculation using Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation

$$eGFR = 141 \times \min(SCr/k,1)^{\alpha} \times \max(SCr/k,1)^{-1.209} \times 0.993^{Age}$$

- multiply by 1.018 for females
- multiply by 1.159 for blacks

- SCr - serum creatinine (mg/dL)
- k is 0.7 for females and 0.9 for males
- α is -0.329 for females and -0.411 for males
- min indicates the minimum of SCr/k or 1
- max indicates the maximum of SCr/k or 1

OTHER INVESTIGATIONS:

ULTRASONOGRAM(USG) ABDOMEN:

Elementary information given by renal USG includes kidney size, cortical echogenicity, parenchymal thickness, cortico-medullary differentiation, presence of cysts (simple, complex), solid lesions and the state of urinary tract.

Renal size in normal individuals is usually in proportion to body height, and normally lies between 9 and 12 cm. In normal persons, the echo consistency of the renal cortex is reduced compared to medulla and the collecting system. In adults the loss of this ‘cortico-medullary differentiation’ is a sensitive but non-specific marker of CKD.

RESULTS

AND

STATISTICAL

ANALYSIS

RESULTS AND STATISTICAL ANALYSIS

A total of 90 subjects were selected as the study group for the present study. This includes 45 cases with CKD and 45 healthy controls.

Levels of blood glucose, serum FGF23, urea, creatinine, albumin, calcium, phosphate, alkaline phosphatase were estimated for all the samples of the study group. Urine albumin was semiquantitatively estimated. eGFR was calculated from the CKD-EPI formula. USG Abdomen was done for both the cases and controls.

Student's t-test, Chi-square test and oneway ANOVA were used for the statistical analysis of data. The datas were expressed in terms of mean and standard deviation. 'p' value less than 0.05 was taken as the significant value. Correlation between the measured parameters were assessed using Pearson's correlation coefficient.

The biochemical values obtained for cases and controls are presented in the master chart I and II respectively.

MASTER CHART FOR CASES - I

SL. No.	AGE	SEX	SYST. BP	DIAST. BP	FBG	UREA	CREAT	CALCIUM	PHOS	ALP	ALBU-MIN	URIC ACID	Na+	K+	FGF23	eGFR	TOTAL KIDNEY VOLUME	URINE ALBUMIN
1	56	F	140	100	118	24	1	10.6	4.1	121	3.4	3.3	144.9	4	87.9	62.9	57.99	NIL
2	40	M	136	90	109	37	1.3	8.7	3.5	102	3.4	3.7	143.9	4.1	83.3	68.3	58.03	NIL
3	65	M	140	100	79	38	1.2	10.3	3	71	2.6	4.3	145.1	3.6	310.9	63.1	61.09	NIL
4	40	F	128	68	98	82	1	10.3	4.1	107	2.6	3.9	145.2	4.3	304.8	70.4	70.99	+
5	68	M	102	78	83	72	2	9.2	2.5	126	2.9	5.3	137.2	4.5	181.8	33.3	66.1	+
6	65	F	120	70	88	91	1.7	10.1	3.9	145	4.1	5.1	142.9	3.7	543.4	31.1	67.75	+
7	61	M	146	94	84	47	2.1	10.3	4	65	2.7	5.1	147	3.4	1263	33	69.87	2+
8	60	M	164	76	83	30	1.3	8.6	3.8	113	3.4	4.8	142.4	2.8	203.3	59.3	34.09	TRACE
9	40	M	148	78	83	73	1.6	8.3	2.7	90	2.9	5.8	142.7	4	263.8	39.9	62	NIL
10	60	M	168	102	54	58	1.9	9.8	4.2	107	4	4.5	165.3	2.9	229.1	37.5	57.38	+
11	73	F	172	84	101	65	2.3	8.8	6.6	99	3	7.5	143.9	4.1	288.8	20.4	46.27	TRACE
12	68	M	142	98	191	44	2.3	10.3	6.2	144	3.2	3.6	157.3	3.2	1676.5	28.1	39	TRACE
13	75	M	140	80	112	65	3.3	8.9	2.8	103	2.6	4.9	151.9	3.7	864.2	17.3	62.9	TRACE
14	45	M	150	98	81	99	2.7	9.9	5.8	94	3.2	5.2	147.1	4.7	270.7	27.2	85.25	NIL
15	40	F	160	90	72	69	2.5	10.5	5.4	210	3.2	4.2	138.4	4.8	681.7	23.3	51.88	+
16	40	F	130	80	64	52	2.4	8.7	6	158	3.4	5.7	149.4	5.2	929.1	24.4	59.92	NIL
17	59	M	132	84	59	75	3.2	9.7	3.2	159	3.4	6.3	143.5	3.7	1272.1	20.1	60.5	TRACE
18	49	M	110	90	110	68	3	10.8	4.1	103	2	5.2	136.2	4.2	725.4	23.3	69.68	+
19	60	M	126	98	100	64	2.5	9.3	4.4	64	4	5.3	136.2	3.9	214.6	26.9	111.12	+
20	53	M	120	90	96	102	4	10.6	4.5	73	3.3	5.1	150.1	3.5	1272.8	16	41.25	TRACE
21	50	F	148	96	274	24	2.8	10.5	4.9	101	3.3	6.6	154.2	3.3	308.9	18.9	56.66	+
22	50	F	160	90	86	77	2.7	10	5	158	2.3	3.8	137.8	3.5	532.5	19.8	35.69	+
23	45	M	138	94	96	54	2.1	10.8	5.2	67	3.8	4.2	137.8	4.5	282.1	27.7	51.33	+

MASTER CHART FOR CASES - I

SL. No.	AGE	SEX	SYST. BP	DIAST. BP	FBG	UREA	CREAT	CALCIUM	PHOS	ALP	ALBU-MIN	URIC ACID	Na+	K+	FGF23	eGFR	TOTAL KIDNEY VOLUME	URINE ALBUMIN
24	70	M	168	70	71	78	2.4	10.5	5.8	67	3.5	7.4	150.2	3.1	738.6	19.8	50.98	+
25	55	M	148	100	109	63	2.7	10.2	5.9	102	3.8	3.9	139.3	4.5	670.5	25.4	56.13	NIL
26	60	M	136	84	106	40	2.8	8.6	6.1	167	1.8	5.9	146.7	3.9	893.2	23.5	49.76	TRACE
27	65	M	130	92	100	82	4.9	8.5	5.8	83	2	4.6	139.4	4.2	970.9	11.5	66.3	2+
28	46	F	126	80	92	96	4	10.7	5.8	103	2.8	6.2	143.3	4.7	991.6	12.6	58.61	NIL
29	55	F	152	82	155	80	3.5	9.2	6.5	171	2.3	4.1	143.6	3.2	1277.9	13.9	62.16	+
30	45	F	160	90	98	85	8.2	10.5	6	121	2.5	6.5	143.1	3.4	1629.1	5.3	72.76	2+
31	45	F	130	90	64	58	3.7	10.2	6.7	102	3.2	3.5	146.8	3.8	1310.9	14	53.94	+
32	48	F	110	80	78	96	4.6	11.7	6.4	92	3.4	4	152.1	4.9	322.8	10.5	43.32	+
33	65	F	136	90	94	103	3.4	9.8	4.5	97	2.5	5.7	147.1	3.8	278.1	13.5	56.5	2+
34	60	M	136	102	116	96	5.3	10.7	6.3	131	3	3.6	147.6	4.3	1032.7	10.8	61.52	+
35	57	M	120	90	147	115	4.3	10.1	4.8	49	2.3	5.9	138.3	4.3	353.1	14.3	63.39	+
36	49	M	160	90	94	106	9	10	4.9	81	2.5	6.2	134.4	4.7	1605.1	6.2	48.64	2+
37	65	F	136	86	159	80	4.4	9.4	6	118	3.3	6.1	153.5	4.7	926.6	9.9	30.31	+
38	62	M	140	88	95	72	4.1	8.8	5.1	181	2.5	3.9	146.9	3.6	1674.7	14.6	52.09	+
39	61	M	162	92	95	128	5.7	10.1	5.4	323	3.3	5.4	139	3.1	300.6	9.9	61.08	NIL
40	40	F	160	70	106	73	3.8	9.9	5.4	126	3	6	152.6	4.3	1285.1	14	53.13	+
41	60	M	156	96	132	128	6.5	9.3	5.5	126	3.3	4.5	145.3	3.9	208.1	8.5	66.34	+
42	45	F	148	78	168	122	4.3	10.4	6	92	2.6	4.5	146.8	5	1091.6	11.7	47.95	+
43	34	M	140	86	96	62	5.6	9.1	6.2	79	2	4.8	152.8	2.9	1342.9	12.2	74.4	3+
44	42	F	152	62	84	65	6.4	10.2	6.4	104	2.6	7.5	138.3	4.6	971.2	7.4	170.76	TRACE
45	35	M	120	70	74	180	8	10.3	6.5	151	2.6	5.7	139.9	4.9	215.6	7.9	58.44	+

MASTER CHART FOR CONTROLS - II

SL. No.	AGE	SEX	SYST. BP	DIAST. BP	FBG	UREA	CREAT	CALCIUM	PHOS	ALP	ALBU-MIN	URIC ACID	Na+	K+	FGF23	eGFR	TOTAL KIDNEY VOLUME	URINE ALBUMIN
1	49	F	106	80	106	30.2	0.7	9.5	2	82	3.9	3.7	135.1	4.3	16.8	101.7	111.78	NIL
2	43	F	140	86	104	15	0.5	10.1	2.4	83	3.7	3.6	134.5	4.9	18	118.5	97.7	TRACE
3	40	F	100	66	56	29	0.4	9.6	2.4	54	5.1	3.8	142.4	3.9	51.4	130.3	95.68	NIL
4	62	M	110	90	83	25	0.7	10.5	3.2	131	3.6	4.3	144.2	3.7	56.2	101.1	93.3	NIL
5	50	M	124	84	86	17.3	0.5	10.6	3.2	64	4.3	4.3	139.3	3.9	39.1	126.4	79.2	NIL
6	36	F	100	70	59	18	0.5	10.9	3.2	94	3.6	3	139.2	4.2	26.8	124.5	82.32	NIL
7	40	M	110	80	89	19	0.6	10.9	3.5	65	3.7	4.7	137.7	4.9	56.7	125.8	85.07	NIL
8	45	F	112	80	83	17	0.7	10	3.5	83	3.6	4.5	135.2	5.2	13.7	104.6	107.4	NIL
9	46	M	150	70	86	15	0.7	10.1	3.6	69	4.2	3.5	136.6	4.4	31.4	113.2	109.67	TRACE
10	40	F	120	80	77	24	0.5	10.7	3.7	81	4.1	3.5	135.1	3.9	35.7	121.1	105.15	TRACE
11	35	M	124	68	72	19	0.6	10.4	3.7	93	3.4	5.3	143.2	5.3	52.1	130.3	86.98	NIL
12	65	M	130	80	107	19	0.8	9.8	3.8	72	3.8	4.1	143.9	3.4	31.6	93.7	104.83	NIL
13	48	M	128	70	132	31	0.7	9.5	3.8	68	4.2	5.6	134.8	4.4	50.9	111.6	99.4	NIL
14	36	M	126	86	124	23	0.8	10.8	3.9	71	2.8	5.1	135.8	3.9	42.6	114.9	91.49	TRACE
15	52	M	130	90	91	16	0.7	10	3.9	78	3.7	4.3	142.6	4.2	53.6	108.5	112.92	NIL
16	25	F	100	60	67	11.2	0.5	10.1	3.9	54	3.9	3.6	138.2	5.2	34.7	134.5	83.99	NIL
17	50	M	108	80	100	25	0.7	10.2	4	78	3.9	2.8	137.5	3.5	43.6	110	101.26	NIL
18	30	F	110	80	98	15	0.5	10.8	4	71	4	3.3	137	3.9	31.6	129.9	89.52	NIL
19	52	M	132	90	87	24	0.7	10.2	4.1	73	3.9	4.2	141.5	4.2	42.9	108.5	89.92	NIL
20	56	M	130	80	85	21	0.7	9.8	4.1	118	3.2	2.5	136.7	4.3	47.8	105.5	101.63	NIL
21	65	M	110	80	117	12	0.8	10.8	4.1	58	4.8	6.1	138.3	5.1	58.9	93.7	89.16	NIL
22	34	F	110	70	68	21	0.5	10.8	4.2	68	3.6	2.8	143.2	3.7	47.8	126.3	101.76	NIL
23	30	F	100	76	78	18	0.5	9.8	4.2	72	4.3	4.5	141.6	3.9	51.9	129.9	104.76	NIL

MASTER CHART FOR CONTROLS - II

SL. No.	AGE	SEX	SYST. BP	DIAST. BP	FBG	UREA	CREAT	CALCIUM	PHOS	ALP	ALBU-MIN	URIC ACID	Na+	K+	FGF23	eGFR	TOTAL KIDNEY VOLUME	URINE ALBUMIN
24	62	M	120	86	89	13	0.6	10.4	4.3	59	3.3	6.2	143.4	3.9	25	107.8	85.06	NIL
25	44	F	120	80	170	15.6	0.5	9.4	4.3	46	3.9	4.7	139.3	5.5	38.8	117.7	105.14	NIL
26	46	M	130	70	136	19	0.7	9.5	4.4	105	3.9	3.1	137	4.2	51.7	113.2	137.31	NIL
27	60	F	130	76	96	26.8	0.6	10.5	4.5	65	4	4.8	139.3	3.5	33.8	122.3	98.15	NIL
28	40	M	116	96	127	22	0.7	9.8	4.5	82	3.5	4.3	137.9	4.2	18.9	118	89.38	NIL
29	56	F	126	74	104	21	0.6	9.3	4.6	56	3.4	6	142.2	4.5	42.8	101.9	99.66	NIL
30	50	M	110	70	68	28	0.6	9.8	4.7	75	4.1	3	144.4	4	43.8	117.2	111.28	NIL
31	38	F	100	80	71	18	0.5	10.3	4.7	67	3.6	3.1	147.8	4.1	42.9	122.8	105.11	NIL
32	42	M	140	80	85	21	0.7	9.6	4.7	65	3.1	4.4	138.4	5.3	48.9	116.4	101.28	NIL
33	45	M	116	66	50	15	0.8	10.8	4.8	101	3.5	2.8	142.4	4.2	52.1	107.9	62.85	NIL
34	52	F	102	74	101	22	0.6	10.3	4.8	76	3	3.2	142.6	4.9	35.6	104.8	94.85	NIL
35	61	F	90	70	81	28	0.6	10.1	4.8	96	3.2	5.3	136.9	5	14.8	123.2	94.15	NIL
36	42	M	130	96	115	18	0.7	9.6	4.8	59	3.7	5.1	145.2	5	28.2	116.4	108.27	NIL
37	42	F	98	70	85	13	0.6	10.6	4.9	45	3.7	3.9	137.5	3.7	42.7	112.4	100.75	NIL
38	54	M	110	76	299	22	0.7	10.2	4.9	92	4.6	3.5	136.8	4.2	52.7	107	86.75	NIL
39	48	M	120	70	85	13	0.8	10.6	4.9	45	3.7	3.9	146.1	4.5	35.6	105.6	88.12	NIL
40	40	F	110	60	88	15	0.7	9.3	5	68	3.5	2.6	138.3	3.4	31.5	108.4	83.05	NIL
41	49	M	164	110	312	14	0.7	10.2	5	76	3.5	4.9	142.5	3.6	35.1	110.8	73.7	TRACE
42	36	F	112	96	94	17	0.7	10.8	5	64	3.4	3.5	143.9	3.8	45.5	111.5	87.13	NIL
43	45	F	126	84	82	17.5	0.7	10.1	5	75	3.7	4.2	145.9	4.6	32.3	104.6	87.99	NIL
44	52	M	136	74	94	33	0.7	10.8	5.1	75	3.5	4.4	135.7	4.6	26.6	108.5	104.88	TRACE
45	54	M	140	80	90	16.1	0.7	9.5	6.7	79	3.5	5.8	142.4	3.7	62	98.2	101.07	+

Table 1

DESCRIPTIVE STATISTICS OF THE STUDY GROUP (n=90)

S.NO	PARAMETERS	CASES(n=45)				CONTROLS (n=45)			
		MIN	MAX	MEAN	SD	MIN	MAX	MEAN	SD
1	Age (years)	34	75	53.9	10.8	25	65	46.4	9.6
2	SBP(mm/Hg)	102	172	141	16.7	90	164	119	15.2
3	DBP (mm/Hg)	62	102	86.5	10.0	60	110	78.5	10.0
4	FBG (mg/dl)	54	274	103.42	38.55	50	312	101.71	49.73
5	Blood Urea (mg/dl)	24	180	75.95	30.41	11.2	30.2	19.83	5.44
6	Sr. Creatinine (mg/dl)	1.0	9.0	3.522	1.920	0.4	0.8	0.64	0.103
7	eGFR(ml/min)	5.3	70.4	23.769	16.97	93.7	134.5	113.80	10.21
8	Sr.Calcium(mg/dl)	8.3	11.7	9.849	0.779	9.3	10.9	10.164	0.492
9	Sr.Phosphate(mg/dl)	2.5	6.7	5.064	1.1786	2.0	6.7	4.196	0.8331
10	Sr.ALP(U/L)	49	323	116.58	46.97	45	131	74.47	17.10
11	Sr.Albumin(g/dl)	1.8	4.1	2.967	0.569	2.8	5.1	3.747	0.445
12	Sr. Uric acid(mg/dl)	3.3	7.5	5.096	1.108	2.5	6.2	4.129	0.983
13	Sr. Sodium (mEq/L)	134.4	165.3	145.05	6.328	134.5	147.8	139.98	3.588
14	Sr.Potassium (mEq/L)	2.8	5.2	3.987	0.628	3.4	5.5	4.282	0.572
15	Sr.FGF23(pg/ml)	83.3	1676.5	730.70	492.72	13.7	62	39.49	12.47
16	eGFR (ml/min)	5.3	70.4	23.769	16.97	93.7	134.5	113.80	10.21
17	Rt.Kidney length	6.2	13.2	8.042	1.191	7.9	12.1	10.151	0.982
18	Rt.Kidney breadth	2.5	9.2	3.697	1.107	3.5	5.6	4.61	0.516
19	Rt kidney Volume	16.74	89.76	30.489	13.616	31.85	59.92	46.824	6.962
20	Lt.Kidney length	5.2	13.5	8.04	1.218	8.5	13	10.33	0.921
21	Lt.Kidney breadth	2.3	6	3.69	0.649	3.1	6.2	4.79	0.508
22	Lt kidney Volume	13.57	81	30.293	10.269	31	80.6	49.415	6.917
23	Total kidneyVolume	30.31	170.76	60.783	21.633	62.85	137.3	96.24	12.446

Table 2

AGE WISE DISTRIBUTION OF THE STUDY GROUP (n=90)

Age group	Cases n (%)	Controls n (%)	Total n (%)
21- 30 years	0 (0)	3 (6.7)	3 (3.3)
31 - 40 years	8 (17.8)	11 (24.4)	19 (21.1)
41 – 50 years	12 (26.7)	17 (37.8)	29 (32.2)
51 – 60 years	12 (26.7)	8 (17.8)	20 (22.2)
Above 60 years	13 (28.9)	6 (13.3)	19 (21.1)
Total	45 (100)	45 (100)	90 (100)

The age group of cases ranged from 34-75 years with a mean age of 53.9±10.8 years and median age of 55 years. The age group of controls ranged from 25-65 years, with the mean age of 46.4±9.6 years and median age of 46 years.

The Chi-square value was 7.715 and the 'p' value between cases and controls was 0.103 and so the difference in the age distribution of cases and controls was not statistically significant and hence they are comparable.

Fig.8 Age distribution of the study population (n=90)

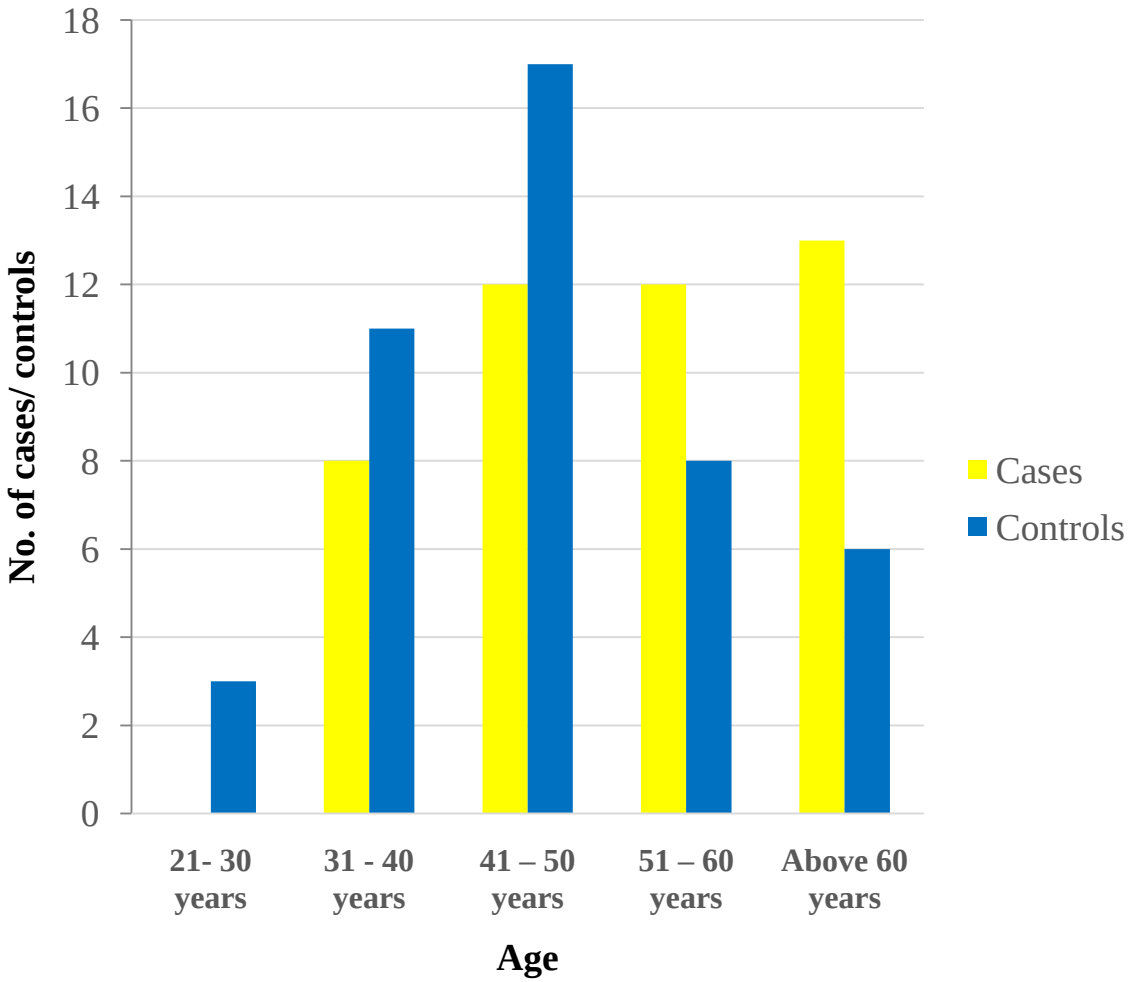


Table 3

GENDERWISE DISTRIBUTION OF THE STUDY GROUP AND STATISTICAL ANALYSIS (n=90)

Gender	Cases n (%)	Controls n (%)	Total n (%)	Statistical Inference
Male	27 (60)	25 (55.6)	52 (57.8)	$X^2 = 0.182$; p value = 0.670
Female	18 (40)	20 (44.4)	38 (42.2)	
Total	45 (100)	45 (100)	90 (100)	

The p value is 0.670(>0.05) and it is observed that the difference was not statistically significant and so in our study, males and females were equally distributed in both cases and controls.

Fig.9 Gender distribution of the study population (n=90)

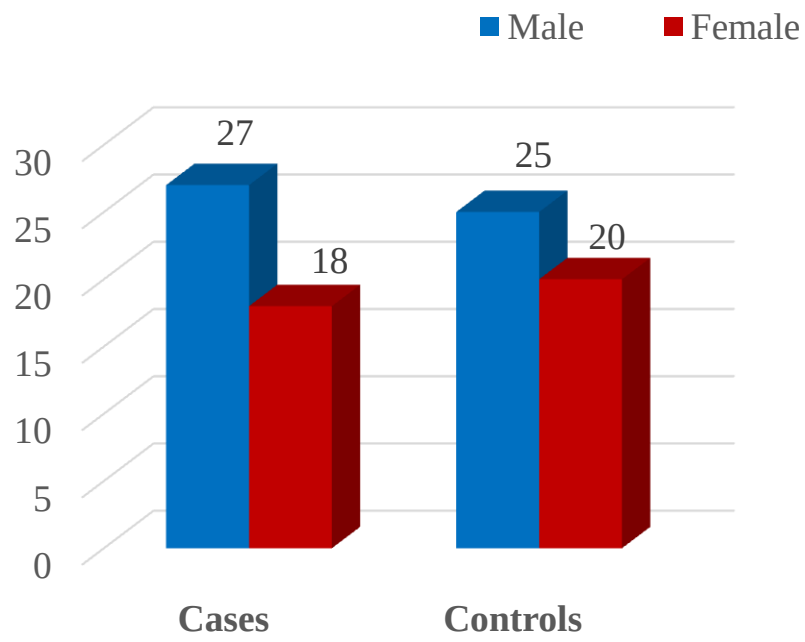


Table 4

COMPARISON OF SERUM FGF23 LEVELS IN THE STUDY GROUP

(n=90)

Serum FGF-23	Cases (n=45)	Controls (n=45)	Student 't' Test p Value
Mean	730.70	39.49	<0.001*
Standard deviation	492.72	12.47	

* Significant at 0.05 level

The mean value of FGF23 in cases was 730.7 ± 492.72 pg/ml was higher than that of the control group whose mean value was 39.49 ± 12.47 pg/ml and this difference was statistically significant($p<0.05$) .

Fig.10 Mean Serum FGF23 levels of the study population (n=90)

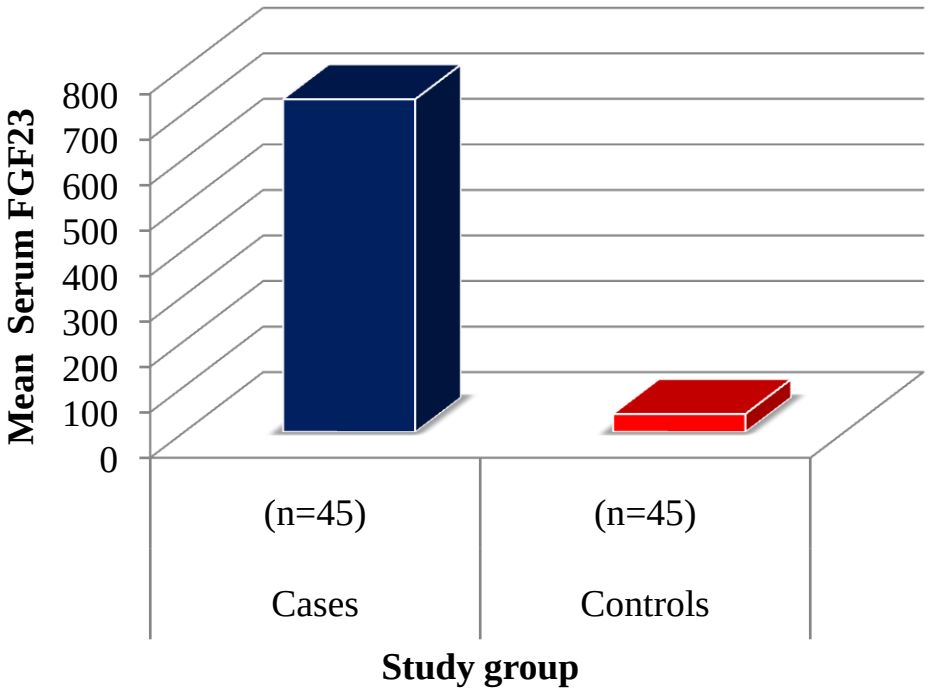


Table 5

GENDERWISE COMPARISON OF SERUM FGF23 IN CASES

(n=45)

S.No	Gender	Serum FGF23 (pg/ml)		Statistical Inference
		Mean	SD	
1	Males(n=27)	708.13	525.06	P=0.70 (p>0.05 is not significant)
2	Females(n=18)	764.55	452.41	

This table shows the gender wise comparison of serum FGF23 levels within cases [males: mean 708.13 ± 525.06; females 764.55 ± 452.41]. There was no significant difference of serum FGF23 levels between males and females in the cases (p = 0.70 ; p>0.05 is not significant).

Fig.11 Genderwise Comparison Of Serum FGF23 in Cases

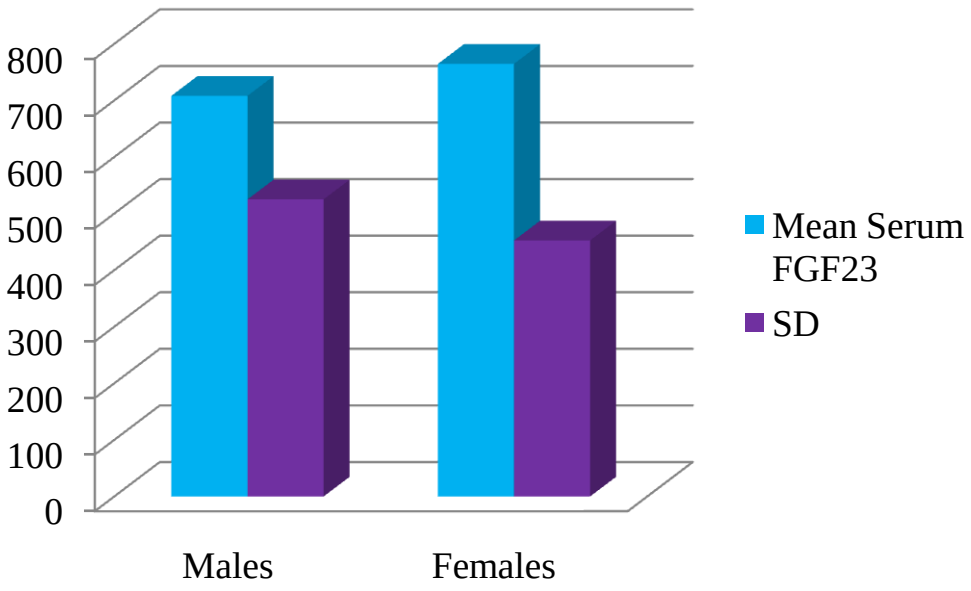


Table 6

COMPARISON OF eGFR LEVELS AMONG CASES AND CONTROLS (n=90)

eGFR levels	Cases (n=45)	Controls (n=45)	Student 't' test p value
Mean	23.769	113.80	<0.001*
SD	16.97	10.21	

* Significant at 0.05 level

The above table shows that cases had very low mean eGFR levels than controls and this difference was statistically significant.

Fig.12 Comparison of eGFR levels and serum FGF-23 among cases and controls (n=90)

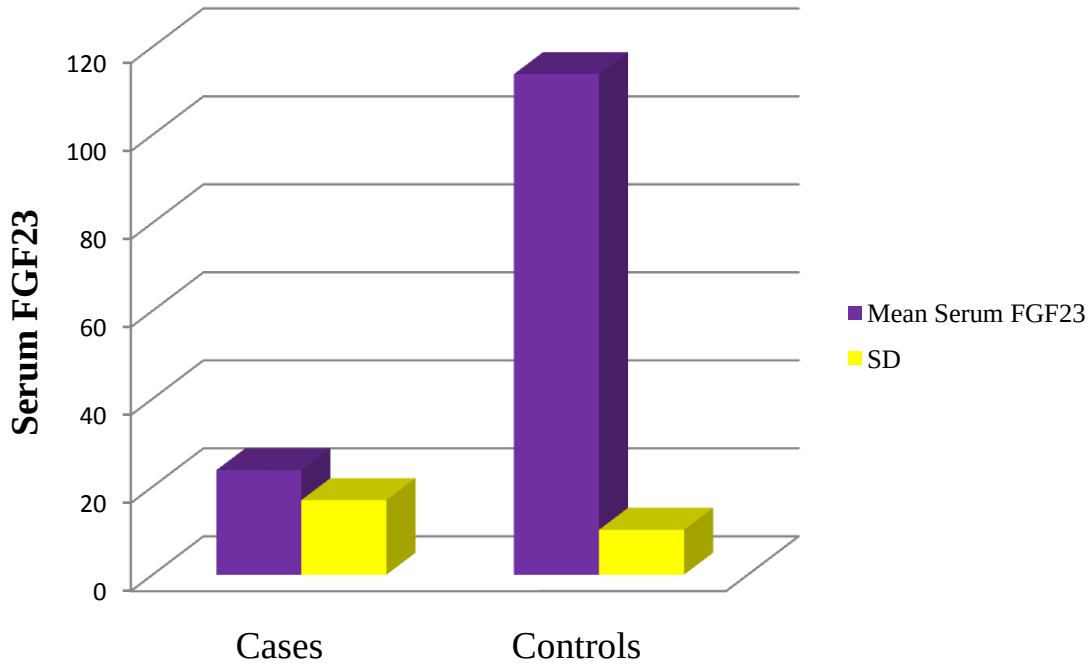


Table 7

DISTRIBUTION OF STUDY POPULATION ACCORDING TO ALBUMINURIA (n=90)

Urine Albumin	Cases n (%)	Controls n (%)	Total n(%)
NIL	9 (20)	38 (84.4)	47 (52.2)
TRACE	8 (17.8)	6 (13.3)	14 (15.6)
1+	22 (48.9)	1 (2.2)	23 (25.6)
2+	5 (11.1)	0 (0)	5 (5.6)
3+	1 (2.2)	0 (0)	1 (1.1)
Total	45 (100)	45 (100)	90 (100)

Chi-square value: 43.353 p value: <0.001

As per the above table, it was observed that albuminuria was both common and severe among cases than controls and this difference was statistically significant.

Fig.13 Comparison of albuminuria among cases and controls (n=90)

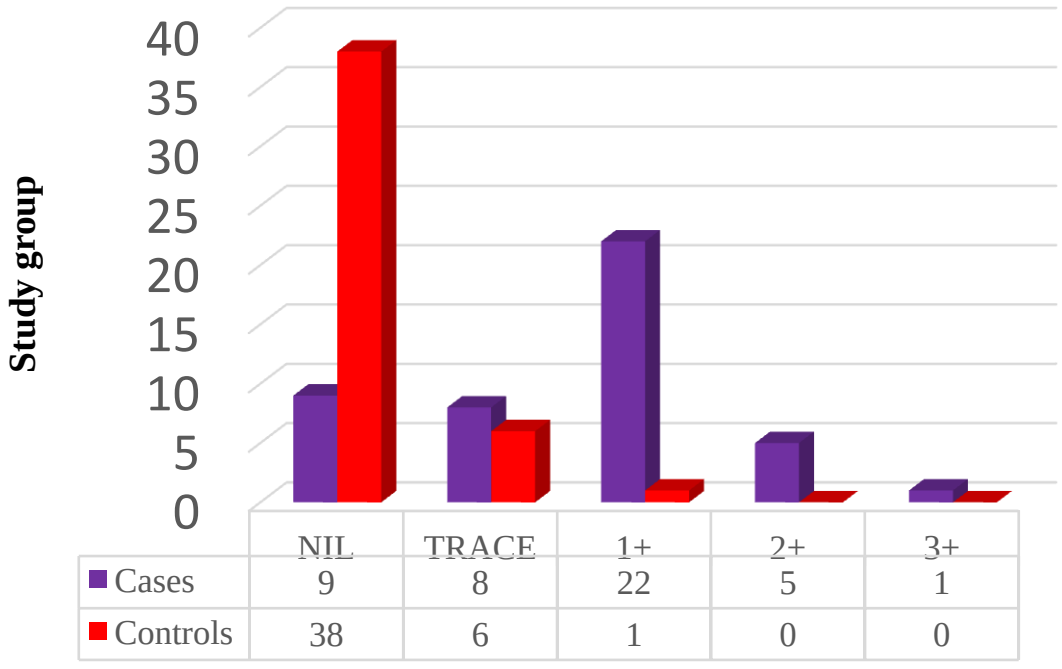


Table 8

DISTRIBUTION OF ALBUMINURIA ACCORDING TO eGFR AMONG CASES AND CONTROLS (n=90)

Parameter	Study Group	eGFR (ml/ min)	Cases		Controls		ANOVA test
			Mean	Standard Deviation	Mean	Standard Deviation	p value
Albuminuria (median and inter-quartile range)	Controls	≥90	---	---	Nil	Nil	<0.001
	Cases	60-89	Nil	Nil to Trace	---	---	
		30-59	1+	Trace to 1+	---	---	
		15-29	Trace	Trace to 1+	---	---	
		<15	1+	1+ to 2+	---	---	

The difference in mean levels of albuminuria between various eGFR groups was statistically significant among the cases.

Table 9

COMPARISON OF SERUM GLUCOSE LEVELS AND RENAL PARAMETERS AMONG CASES AND CONTROLS (n=90)

Parameter		**Cases (n=45)**	**Controls (n=45)**	**Student 't' test p value**
Fasting blood glucose (mg%)	Mean	103.42	101.71	0.856
	Standard deviation	38.55	49.73	
Blood urea (mg%)	Mean	75.95	19.83	<0.001*
	Standard deviation	30.41	5.44	
Serum creatinine (mg%)	Mean	3.522	0.640	<0.001*
	Standard deviation	1.920	0.103	
Serum Uric acid (gm %)	Mean	5.096	4.129	<0.001*
	Standard deviation	1.108	0.983	

* Significant at 0.05 level

The above table shows a significantly higher blood urea , serum creatinine and serum uric acid levels in the cases than controls ($p<0.05$). There was no significant difference in the Fasting blood glucose between the two groups.

Table 10

COMPARISON OF VARIOUS SERUM BIOCHEMICAL PARAMETERS AMONG CASES AND CONTROLS (n=90)

Parameter		Cases (n=45)	Controls (n=45)	Student 't' test p value
Serum calcium (mg%)	Mean	9.849	10.164	0.024*
	Standard deviation	0.779	0.492	
Serum Phosphate (mg%)	Mean	5.064	4.196	<0.001*
	Standard deviation	1.1786	0.8331	
Serum Alkaline phosphatase (U/L)	Mean	116.58	74.47	<0.001*
	Standard deviation	46.97	17.10	
Serum albumin (gm %)	Mean	2.967	3.747	<0.001*
	Standard deviation	0.569	0.445	
Serum Sodium (mEq/L)	Mean	145.05	139.98	<0.001*
	Standard deviation	6.328	3.588	
Serum Potassium (mEq/L)	Mean	3.987	4.282	0.022*
	Standard deviation	0.628	0.572	

The observation is that controls had high levels of serum calcium, phosphate and albuminthan cases and this difference in the mean levels were statistically significant. Also cases had high levels of serum alkaline phosphatase, sodium and potassium than controls and this difference in the mean levels were also statistically significant.

Table 11

COMPARISON OF CREATININE IN RELATION TO eGFR AMONG CASES AND CONTROLS (n=90)

Study group	eGFR (ml/min)	Creatinine(mg/dl)		Statistical Inference
		Mean	SD	
Controls (n=45)	≥90	0.6	0.1	***p<0.001***; p<0.05 Significant
Cases (n=45)	60-89 (n=4)	1.1	0.2	
	30-59 (n=6)	1.8	0.3	
	15-29 (n=16)	2.7	0.5	
	<15 (n=19)	5.2	1.7	

This table shows the comparison of serum Creatinine levels in the study group in relation to eGFR. It was observed a significant progressive increase of Creatinine values as the renal function decreases in cases. Serum Creatinine levels (p<0.05) were within normal reference range for controls.

Fig.14 Comparison of Creatinine in relation to eGFR in the study group

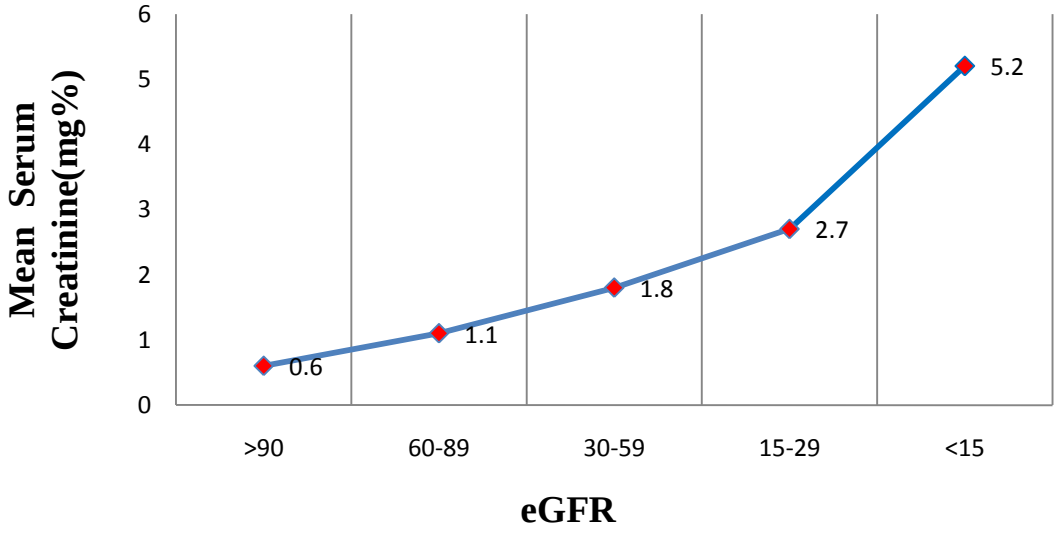


Table 12

COMPARISON OF FGF23 IN RELATION TO eGFR AMONG CASES AND CONTROLS (n=90)

Study Group	eGFR(ml/min)	MeanFGF-23 levels	Std. Deviation	95% Confidence Interval for Mean		Statistical Inference
				Lower Bound	Upper Bound	
CONTROLS (n=45)	≥90	39.491	12.4712	35.744	43.238	p=0.014; P<0.05 Significant
CASES (n=45)	60-89 (n=4)	196.725	128.3540	-7.515	400.965	
	30-59 (n=6)	447.400	420.9303	5.66	889.13	
	15-29 (n=16)	726.356	421.3157	501.85	950.86	
	<15 (n=19)	936.242	508.7571	691.02	1181.45	

ANOVA test was applied to test the difference in mean FGF-23 levels between the groups .

ANOVA test

p value	0.001*
F statistic	33.879
Degree of freedom	4

Comments:

1. ANOVA test shows that there was a statistically significant difference in the mean FGF-23 levels between the controls and patients with various stages of CKD according to eGFR.

2. The table also shows that the severity of CKD increases with increase in mean FGF-23 levels and vice versa.
3. Hence, FGF-23 levels were clearly high in end stages of CKD (adverse outcome) and so FGF23 could predict the progression of CKD or deterioration of GFR among CKD patients.

Fig.15 Comparison of serumFGF-23 according to eGFR levels among study population (n=90)

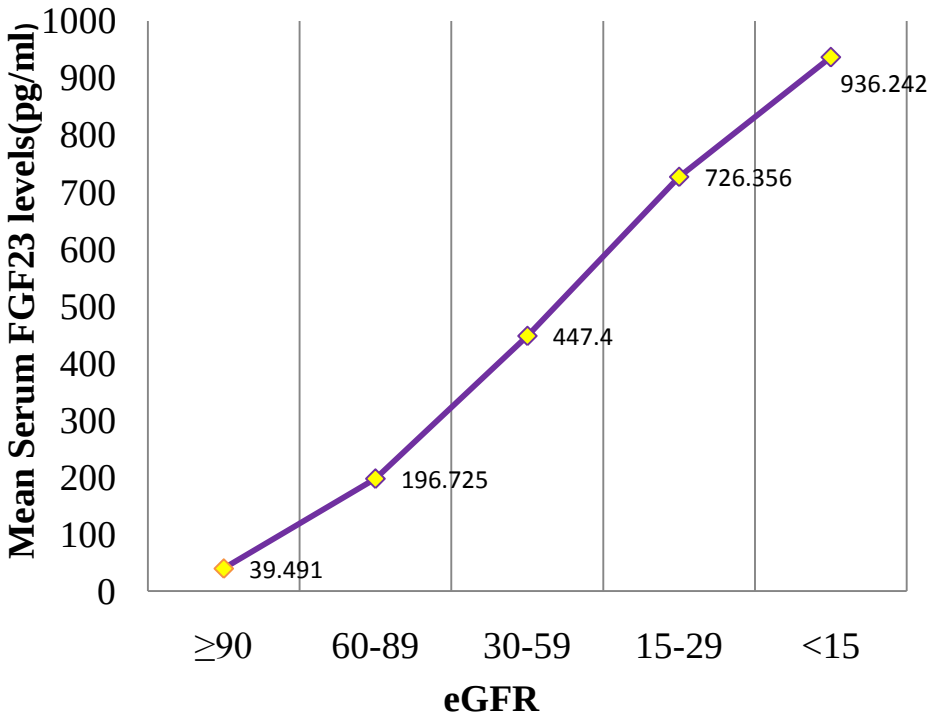


Table 13

COMPARISON OF SERUM PHOSPHATE LEVELS IN RELATION TO eGFR AMONG CASES AND CONTROLS (n=90)

Study Group	eGFR (ml/min)	n	Mean phosphate levels	Std. Deviation	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
Controls (n=45)	≥90	45	4.196	0.8331	3.945	4.446
Cases (n=45)	60-89	4	3.675	0.5315	2.829	4.521
	30-59	6	3.517	0.7250	2.756	4.278
	15-29	16	5.119	1.0889	4.539	5.699
	<15	19	5.800	0.6429	5.490	6.110

ANOVA test was applied to test the difference in mean phosphate levels between the groups.

ANOVA test

p value	<0.001*
F statistic	17.724
Degree of freedom	4

ANOVA test shows that there was a statistically significant difference in the mean phosphate levels between the cases and the stages of CKD according to eGFR. Serum phosphate levels were initially decreased but in the later stages of CKD, as the eGFR declines further, there was a rising trend in serum phosphate levels.

Fig.16 Comparison of serum phosphate levels according to eGFR among study population (n=90)

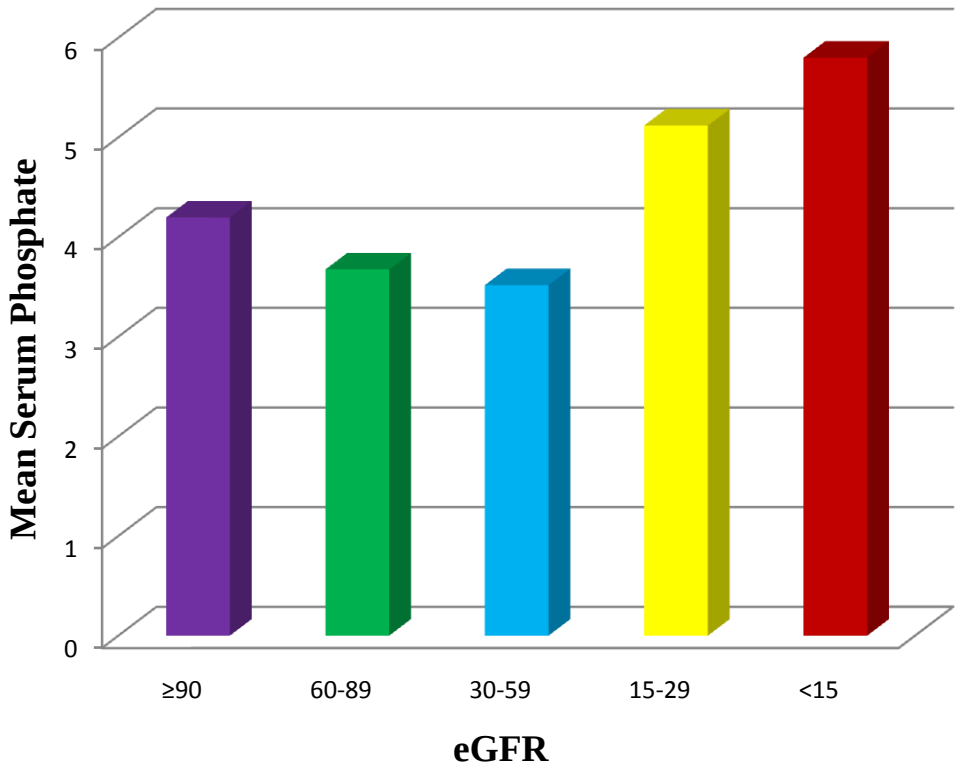


Table 14

DISTRIBUTION OF VARIOUS PARAMETERS ACCORDING TO eGFR GROUPS AND AMONG CASES AND CONTROLS (n=90)

Parameter	Study Group	eGFR (ml/min)	Cases		Controls		ANOVA test
			Mean	Standard Deviation	Mean	Standard Deviation	p value
Serum FGF-23	Controls	≥90	.	.	39.5	12.5	***0.014***
	Cases	60-89	196.7	128.4	.	.	
		30-59	447.4	420.9	.	.	
		15-29	726.4	421.3	.	.	
		<15	936.2	508.8	.	.	
Total kidney Volume	Controls	≥90	.	.	96.24	12.45	0.916
	Cases	60-89	62.03	6.15	.		
		30-59	59.53	13.23	.	.	
		15-29	58.02	18.65	.	.	
		<15	63.24	28.02	.	.	
Serum Sodium	Controls	≥90	.	.	140.0	3.6	0.970
	Cases	60-89	144.8	0.6	.	.	
		30-59	146.3	9.8	.	.	
		15-29	145.0	6.8	.	.	
		<15	144.8	5.6	.	.	
Serum Potassium	Controls	≥90	.	.	4.3	0.6	0.294
	Cases	60-89	4.0	0.3	.	.	
		30-59	3.6	0.7	.	.	
		15-29	4.0	0.6	.	.	
		<15	4.1	0.7	.	.	
Blood Glucose	Controls	≥90	.	.	102	50	0.426
	Cases	60-89	101	17	.	.	
		30-59	79	12	.	.	
		15-29	108	54	.	.	
		<15	108	30	.	.	
Serum Urea	Controls	≥90	.	.	19.8	5.4	<***0.001***
	Cases	60-89	45.3	25.3	.	.	
		30-59	61.8	21.6	.	.	
		15-29	64.9	20.0	.	.	
		<15	96.2	29.6	.	.	
Serum Creatinine	Controls	≥90	.	.	0.6	0.1	<***0.001***
	Cases	60-89	1.1	0.2	.	.	
		30-59	1.8	0.3	.	.	
		15-29	2.7	0.5	.	.	
		<15	5.2	1.7	.	.	

Parameter	Study Group	eGFR (ml/min)	Cases		Controls		ANOVA test
			Mean	Standard Deviation	Mean	Standard Deviation	p value
Serum Calcium	Controls	≥90	.	.	10.2	0.5	0.481
	Cases	60-89	10.0	0.9	.	.	
		30-59	9.4	0.8	.	.	
		15-29	9.9	0.8	.	.	
		<15	9.9	0.8	.	.	
Serum Phosphate	Controls	≥90	.	.	4.19	0.83	***<0.001***
	Cases	60-89	3.67	0.531	.	.	
		30-59	3.51	0.725	.	.	
		15-29	5.11	1.088	.	.	
		<15	5.80	0.642	.	.	
Serum Alkaline phosphatase	Controls	≥90	.	.	74	18	0.809
	Cases	60-89	100	21	.	.	
		30-59	108	28	.	.	
		15-29	117	44	.	.	
		<15	123	58	.	.	
Serum Albumin	Controls	≥90	.	.	3.7	0.4	0.064
	Cases	60-89	3.0	0.5	.	.	
		30-59	3.3	0.6	.	.	
		15-29	3.1	0.6	.	.	
		<15	2.7	0.4	.	.	
Serum Uric Acid	Controls	≥90	.	.	4.1	1.0	0.099
	Cases	60-89	3.8	0.4	.	.	
		30-59	5.1	0.4	.	.	
		15-29	5.3	1.2	.	.	
		<15	5.2	1.1	.	.	
Urine Albumin	Controls	≥90	.	.	Nil	Nil	***<0.001***
	Cases	60-89	Nil	Nil to Trace	.	.	
		30-59	1+	Trace to 1+	.	.	
		15-29	Trace	Trace to 1+	.	.	
		<15	1+	1+ to 2+	.	.	

The difference in mean levels of serum FGF-23 levels, Blood Urea, Serum creatinine, serum phosphate and albuminuria between various eGFR groups were statistically significant ($p<0.05$) among the cases.

TABLE 15

COMPARISON OF MEAN LEVELS OF SERUM FGF23 AND SERUM PHOSPHATE IN RELATION TO eGFR

Parameter	**Controls**	**Cases**			
eGFR(ml/min)	**≥90**	**60-89**	**30-59**	**15-29**	**<15**
Mean Serum FGF23	39.491	196.725	447.4	726.356	936.242
Mean Serum Phosphate	4.196	3.675	3.517	5.119	5.8

It was observed that, with decreasing eGFR in CKD, the levels of serum FGF23 increase prior to increasing serum phosphate and this increase in serum FGF23 levels were observed as early as stage 2 of CKD (eGFR of 60-89 ml/min).

TABLE 16
PEARSON CORRELATION MATRIX BETWEEN SERUM FGF-23 AND VARIOUS PARAMETERS AMONG CASES AND CONTROLS

S.No	Parameters	Cases (n=45)		Controls (n=45)	
		Pearson Correlation	p value	Pearson Correlation	p value
1	eGFR	***-0.484***	***0.001****	-0.048	0.752
2	Total Kidney volume	-0.046	0.765	-0.010	0.948
3	Serum Sodium	0.171	0.262	0.260	0.084
4	Serum Potassium	-0.104	0.495	-0.147	0.337
5	Blood urea	-0.005	0.972	-0.049	0.750
6	Serum creatinine	***0.416***	***0.004****	0.090	0.556
7	Serum calcium	0.035	0.822	0.050	0.743
8	Serum phosphate	***0.348***	***0.019****	0.200	0.187
9	Serum alkaline phosphatase	0.063	0.682	0.071	0.643
10	Serum albumin	***-0.342***	***0.022****	0.234	0.121
11	Serum uric acid	0.054	0.727	0.054	0.727
12	Urine albumin	0.291	0.052	-0.145	0.341

*Significant at 0.05 level

Comments:

1. Among cases, Pearson correlation between serum FGF-23 levels and eGFR, serum albumin were statistically significant and had a negative inverse correlation i.e. Rise in serum FGF-23 levels had a corresponding fall in eGFR and serum albumin.

2. Among cases, Pearson correlation matrix between serum FGF-23 levels and serum creatinine, serum phosphate were statistically significant and had a positive direct correlation i.e. Rise in serum FGF-23 levels had a corresponding rise in serum creatinine and phosphate.

3. Among controls, there were no statistically significant correlation between serum FGF-23 levels and other parameters.

DISCUSSION

DISCUSSION

In the present study serum FGF23 concentrations were found to be significantly increased in patients with CKD (mean 730.70 ± 492.72) when compared to the control group (mean 39.49 ± 12.47).

When patients in different stages of CKD were compared, serum FGF23 levels were found to be progressively increased from stage 2 to stage 5 in comparison with the control group. Serum FGF23 levels were inversely correlated with eGFR. This observation showed that increase in serum FGF23 develops relatively in the early stages of CKD. These findings are in accordance with the study of Larsson et al which reported a progressive increase in serum FGF23 levels over the spectrum of CKD[63].

Also, a strong negative correlation was observed between serum FGF23 and eGFR levels ($r = -0.484$, $p < 0.001$ significant) which shows that serum FGF23 levels increase with decreasing eGFR levels in accordance with the study conducted by Takayuki Hamano et al[70].

In the present study, a significantly lower levels of eGFR in CKD cases were observed when compared to controls [mean value: cases 23.769 ± 16.97; controls 113.8 ± 10.21, p value < 0.001] .

It was also observed that serum FGF23 levels were significantly higher in all age groups and in both genders in cases [males: mean 708.13 ± 525.06; females 764.55 ± 452.41] which indicates that age and gender does not have an impact on serum FGF23 levels in accordance with the study conducted by Danilo Fliser et al[71].

The higher levels of serum FGF23 in CKD may be due to hyperphosphatemia, or a novel molecule that stimulates FGF23 secretion or due to low Klotho expression states as in Klotho deficiency[67].

The mineral metabolic changes in CKD are described as CKD-MBD which includes hyperphosphatemia, hypercalcemia, increased PTH levels and low calcitriol levels[72]. The severity of CKD-MBD is linked with an increased mortality in these patients.

FGF23 acts on FGFR- Klotho receptor complex to cause phosphaturia and decreases calcitriol synthesis. Both high phosphate and 1,25-dihydroxy Vitamin D causes stimulation of FGF23 production. So, lack of FGF23 leads to hyperphosphatemia and high calcitriol levels with a risk of extraosseous calcification[73].

In relation to FGF23, PTH directly modifies phosphate and calcitriol levels which eventually leads to an effect on FGF23 secretion. Also, serum FGF23 levels increase early in the course of CKD even before PTH is increased. In CKD, as there is a progressive loss of nephrons, both PTH and FGF23 becomes non-operative and it is then, serum phosphate levels begin to rise. When FGF23 is elevated, it indicates an already existing inadequate phosphate control[74].

Serum FGF23 levels increase much earlier in the course of CKD. Thus FGF23 would be a more reliable indicator of phosphate burden rather than a single measurement of serum phosphate. As of now, the existing creatinine

based estimations of GFR, used for the assessment of kidney function do not always predict CKD at an early stage[75].

These results of the present study are in accordance with that of the previous studies suggesting increased serum FGF23 levels which occur even in early stages of CKD even before a rise in serum phosphate. Serum phosphate begins to rise from stage 4 of CKD (Mean 5.11±1.088 SD), whereas serum FGF23 levels start increasing as early as Stage 2 of CKD (Mean 196.7±128.4 SD). The difference in mean levels of Serum FGF-23 levels, serum phosphate between various eGFR groups were statistically significant ($p<0.05$) among the cases.

Among cases, Pearson correlation matrix between serum FGF-23 levels and serum phosphate were statistically significant and had a positive direct correlation i.e. Rise in serum FGF-23 levels had a corresponding rise in serum phosphate ($r = 0.348$, p value = 0.019, significant at 0.05 level). These results are similar to the work done by Thomas J Weber et al[76].

Phosphate, even in the upper limit of normal range, is a potential toxin in CKD patients leading to adverse outcome with increased cardiovascular morbidity and mortality[77].Excessive dietary phosphate directly impairs renal function by inflicting tubulointerstitial damage[78]. High phosphate forms insoluble crystals in the tubular fluid together with calcium, and these crystals lead to tubular injury and progression of CKD. Calcium-phosphate crystals also affect vascular smooth muscle cell function and cause vascular calcification[79].

Taken together the results of the present study suggest that serum FGF23 is an early marker of progression of CKD and its increased levels in serum helps in the early identification of CKD-MBD in patients with CKD. The study also suggests that higher the level of serum FGF23, higher is the severity of CKD. Hence serum FGF23 could be considered as a early marker of progression in CKD patients and in the prevention of complications such as CKD-MBD in the initial stages of CKD.

CONCLUSION

CONCLUSION

- The present study demonstrated that serum FGF23 levels were significantly increased in patients with CKD. This increase in serum FGF23 levels were progressive from the early stages to the late stages of CKD .
- The increased levels of serum FGF23 in CKD may be due to hyperphosphatemia or a novel molecule that stimulates FGF23 secretion or due to low Klotho expression states as in Klotho deficiency.
- Also, Serum PTH increases FGF23 transcription either directly or by increasing bone remodeling and release of local bone factors, such as low-molecular weight FGFs which stimulates the osteocytes to synthesize FGF23.
- FGF23 is involved in the regulation of calcium-phosphate metabolism and thus it is also involved in the mineral metabolic disorders implicated in CKD-MBD.
- Serum FGF23 may be considered as an early marker of progression of CKD. Higher the serum FGF23 levels in CKD, more severe is the disease.

LIMITATIONS OF THE STUDY

- Small sample size of the study population.
- Estimation of other biochemical parameters such as Serum PTH and Vitamin D would have helped better in assessing the disease process of CKD-MBD.
- Correlation of Serum FGF23 with an already established marker of CKD such as Cystatin C would have helped better in assessing the severity of CKD.

SCOPE FOR FUTURE STUDY

- Since current treatment guidelines for CKD recommend to treat the accompanying hyperphosphatemia in advanced stages of CKD , FGF23 screening could thus identify more patients with normal serum phosphate levels at an earlier stage that wouldbenefit from phosphorous related therapies.
- As our knowledge expands regarding the functions of FGF23, assessment of Serum FGF23 would be an important diagnostic marker as well as therapeutic target for the management of CKD-MBD.

ANNEXURES

BIBLIOGRAPHY

BIBLIOGRAPHY

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PROFORMA

STUDY OF SERUM FGF-23 LEVELS IN CHRONIC KIDNEY DISEASE

Name: **Age:** **Sex:**

IP.No: **DOA:** **DOD:**

Complaints **:** Oliguria/Nocturia/Dysuria/Pedal Edema/Anasarca/ Fever/ Weight Loss

Past History **:** HT/ DM/Tuberculosis/Cerebrovascular disease/liver disease/ Rheumatoid arthritis

Personal History **:** Diet/Tobacco/Alcohol/Cigarette use

Family History **:** HT/ DM/ Renal disease

Drug History **:** Drugs (lipid lowering drugs, calcium/ phosphate binders) / renal replacement therapy

General Examination **:** **HR :** **BP :**

Systemic Examination **:** **CVS:** **CNS:**

RS: **ABD:**

Provisional Diagnosis :

Investigations

Serum FGF-23

Blood Glucose(Fasting):

Blood Urea

Serum Creatinine

Serum Uric acid

Serum Electrolytes – Sodium , Potassium

Serum Albumin

Serum Calcium

Serum Phosphorus

Serum Alkaline Phosphatase

Urine Albumin

eGFR Calculation by CKD-EPI Formula

USG Abdomen

CONSENT FORM

I _____ hereby give consent to participate in the **"STUDY OF SERUM FIBROBLAST GROWTH FACTOR(FGF-23) LEVELS IN CHRONIC KIDNEY DISEASE"** conducted by **Dr.PRIYA.A,** Post graduate in Department of Biochemistry, K.A.P.V Govt.Medical College, Trichy and to use my personal clinical data and result of investigation for the purpose of analysis and to study the nature of disease. The study has been explained to me clearly. I understand that there are no risks involved in the study. The data obtained by the study may be used for research and publication.

Place : TRICHY Signature of participant

Date :